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THE UNIVERSITY OF ALBERTA

# ENERGETIC EFFICIENCY OF CIMATEROL-TREATED LAMBS

by

WILLIAM ROBERT CAINE

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

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IN

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled ENERGETIC EFFICIENCY OF CIMATEROL-TREATED LAMBS submitted by WILLIAM ROBERT CAINE in partial fulfilment of the requirements for the degree of MASTER OF SCIENCE in ANIMAL NUTRITION.

J R Thompson for G W Mathison

Supervisor

R J Christopherson

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Date October 13, 1989

## DEDICATION

To my wife, Betty and children, Jill, Jane and Jonathan for their perseverance and support throughout my studies.

## ABSTRACT

1. The effect of dietary cimaterol on feed efficiency, liver oxygen consumption, carcass characteristics and energetic efficiency was evaluated using fifteen wether lambs matched by weight into control, cimaterol and pair-treated groups. The diet consisted of sun-cured alfalfa pellets and barley grain fed to the lambs for 25 d at a low (L)-level (491 KJ metabolizable energy intake [MEI]/$kg^{0.75}$ per d), followed by 50 d at a high (H)-level (911 KJ MEI/$kg^{0.75}$ per d) of feed intake. The control and cimaterol groups received a corresponding 0 and 10 mg cimaterol/kg of feed at both dietary levels. Pair-treated lambs received no cimaterol during the L-level but were fed 10 mg cimaterol/kg of feed during the H-level.

2. When cimaterol was included in the diet average daily gain tended ($P<0.1$) to increase at both feeding levels.

3. The proportion of digestible energy lost as methane increased ($P<0.05$) 0.15 and 0.22 for cimaterol lambs in comparison with the control group at the L-level and with the pair-treated lambs at the H-level, respectively. Retention of energy as protein (KJ/$kg^{0.75}$ per d) was increased ($P<0.05$) in cimaterol (124.1)* and pair-treated (113.7) compared to control (98.9) lambs, at the H-level.

4. Heat production (KJ/$kg^{0.75}$ per d) was 467 and 462 at the L- and 628 and 605 at the H-level for control and cimaterol lambs, respectively ($P=0.57$). Pair-treated lambs had a heat production of 606 KJ/$kg^{0.75}$ per d at the H-level. Hourly and daily mean (24 h)

heart rate and rectal temperature were not different ($P>0.1$) between the groups.

5. The $Na^+,K^+$-ATPase (EC 3.6.1.3) -dependent oxygen consumption rate of liver tissue biopsies taken from lambs the day before slaughter was reduced ($P<0.1$) by 0.2 for cimaterol and 0.1 ($P>0.1$) for pair-treated compared with control lambs.

6. Carcass weight as a proportion of liveweight, area of the *Longissimus dorsi* muscle and weights of the *psoas major*, *gastrocnemius* and *semitendinosus* muscles were increased ($P<0.05$) and crude fat content in muscle tissue samples was reduced ($P<0.05$) by cimaterol treatment.

7. Maintenance requirement was not different ($P=0.69$) between control and cimaterol lambs. Corresponding efficiency of utilization of MEI above maintenance for gain was 0.57 and 0.67 ($P=0.36$). Estimated energy costs of tissue deposition (KJ MEI/g) for control and cimaterol lambs were respectively, 59.9 and 38.9 for protein ($P=0.27$) and 44.4 and 51.1 for fat ($P=0.41$).

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# I. GENERAL INTRODUCTION

## A. METABOLISM AND GROWTH IN ANIMALS

Maintenance and growth. The way in which animals and man assimilate dietary energy and the efficiency with which it is used for productive functions such as egg production, lactation or growth serves as a primary impetus for metabolic research. Classically, animal scientists have separated metabolizable energy intake (MEI), the dietary intake energy available for metabolic processes after correcting for losses in faeces, urine and gas, into maintenance and productive functions. The maintenance requirement is defined as the dietary energy intake at which an animal's body attains energy equilibrium. In metabolic terms, the maintenance requirement is the thermogenesis comprised of the heat increment of feeding at zero energy balance (production of heat due to the ingestion of food) plus basal metabolism. The basic premise for a productive component of energy balance requires that an animal's MEI must exceed a requirement for body maintenance, the remaining energy is then available for productive functions such as growth.

These arbitrary designations have been criticized as an oversimplification of the dynamics of metabolism because they imply separate metabolic processes may be associated with maintenance and growth (Baldwin et al. 1985 and Milligan and Summers, 1986). Nevertheless, the separation of MEI into maintenance and productive functions continues to be a useful convention for the study of bioenergetics (Garrett, 1980), providing much of the information currently being used to develop kinetic modelling systems based on

metabolic interactions (Lobley, 1986) and remains the technical basis for determining the energy requirements of ruminants (Agricultural Research Council [ARC], 1980 and National Research Council, 1985).

Metabolic body size and energy expenditure. As it pertains to a basal metabolic rate, energy expenditure is often expressed in terms of a scaling coefficient which relates a 0.75 exponent of body weight, usually called metabolic body size ($kg^{0.75}$) to the metabolism within and between animal species (Kleiber, 1947). Validation of $kg^{0.75}$ as a scaling factor to describe the energetic relationship between animals has been questioned. Heusner (1985) reviewed interspecies animal data and concluded the 0.75 power was a statistical artifact and suggested that a 0.67 exponent of body weight as a function of animal surface area was biologically more appropriate. In a review of the literature concerning the resting energy expenditure of various species, Thonney et al. (1976) claimed that exponents determined by regression analyses ranged from 0.37 to 0.74 and they concluded that the $kg^{0.75}$ factor was an appropriate parametric scaler when used for interspecies comparisons of basal energy expenditures. Hence, it would be satisfactory for estimating the maintenance requirements of mature animals of most domestic species (Thonney et al. 1976). On the other hand, in growing animals where accretion of tissue and the associated metabolic activities are changing proportionally with the rate of protein and fat deposition, the concept of maintenance and use of $kg^{0.75}$ is less certain. Nevertheless, the 0.75 power was adopted in 1964 at the Third Symposium on Energy Metabolism, as a reference for

comparative studies relating metabolism to body weight (Kleiber, 1964).

Maintenance requirements of growing lambs fed a number of forage diets were predicted with reasonable confidence to be between 231 and 408 $KJ/kg^{0.75}$ per d (Thomson et al. 1979). The energetic efficiency of MEI utilization to fulfill the maintenance requirement of ruminants is relatively constant at about 0.6 to 0.7, although this is dependent to some extent on the quality of the diet (ARC, 1980). However, Fattet et al. (1984) studying protein supplementation in underfed sheep had difficulty in distinguishing MEI utilization for maintenance or growth. Webster (1981) has suggested that energy expenditure is reasonably measured on the basis of lean body mass, which is comprised of metabolically more active tissues. His conclusion was that fat makes a relatively small contribution to basal metabolism, therefore metabolic rate expressed as a 0.75 exponent of lean body weight largely removed the variability within species involved in different physiological activities.

Components of thermogenesis. Basal metabolism, the energy expenditure taking place within the animal body which excludes the metabolic activity asssociated with tissue accretion has been categorically divided into service functions and cell maintenance activities (Baldwin et al. 1980). The service functions considered to be organ (heart, liver, gut, etc.) work accounts for an estimated 0.36 to 0.50 of energy expenditure for animal maintenance. A further 0.40 to 0.56 of energy expended due to metabolic activities such as ion transport and protein and lipid turnover for cellular

maintenance. Energetic costs associated with productive functions can be sub-divided into the energy incorporated as the substrates which actually form accreted tissues and the energy expended by oxidation of metabolites to fulfill the metabolic activity required for growth (Millward et al. 1976). Baldwin et al. (1980) has suggested that improvement in the productive efficiency of livestock may be achieved by changing energy expenditure for cellular maintenance through the reduction of the energy costs of protein turnover and ion transport. Research directed towards the energetic costs of protein metabolism in pigs (Reeds et al. 1980) and steers (Lobley et al. 1987) and $Na^+,K^+$ ion transport in muscle (Gregg and Milligan, 1982), liver (McBride and Milligan, 1985a) and duodenal (McBride and Milligan, 1985b) tissues of sheep indicate that the manipulation of these metabolic activities could indeed result in an improvement of animal productive efficiency.

Particularly interesting is the estimate of 0.20 to 0.25 of total energy expenditure in animals associated with the transmembrane transport of $Na^+$ and $K^+$ ions (Milligan and Summers, 1986). Apparently, $Na^+,K^+$-ATPase-dependent respiration varies substantially as a component of in vitro oxygen consumption in different tissues of individuals in various physiological states, therefore it plays a central role in determining the maintenance requirement of animals (Milligan and McBride, 1985). Moreover, regression of $Na^+,K^+$-ATPase-dependent respiration against protein synthesis showed a close linear relationship and accounted for 0.22 to 0.25 of oxygen consumption in in vitro preparations of intercostal and sartorius muscles of growing pigs (Adeola et al.

1989). This suggests that $Na^+$ and $K^+$ ion transport also makes an important contribution to the energy expenditure associated with productive functions like carcass growth.

Central to the differences in energy expenditures of animals is the estimated 0.4 of thermogenesis contributed by internal organs like the liver and gut (Webster, 1980). Ferrell et al. (1986) have shown that variation in fasting heat production (FHP) and presumably maintenance energy costs of growing lambs are altered by prior levels of nutrition and these differences can be highly correlated to changed weights of metabolically intense organ tissues such as the liver. Furthermore, while the weights of these tissues are related to level of dietary intake and FHP they are also functions of the animal's body size and level of production (Koong et al. 1985).

Estimating energetic efficiency in livestock. Generally, two main approaches have been taken for the study of bioenergetics in domestic livestock. The first approach determines largely theoretical estimates of the efficiencies of metabolic and physiological processes, based on stoichiometric calculations from balanced equations for the formation of intermediates involved in the biochemical pathways associated with energy transformations (Baldwin, 1968 and Milligan, 1971). Theoretical efficiencies of growth based on these calculations range from 0.75 to 0.85 in rats and 0.70 to 0.80 in ruminants, depending on the metabolic pathways chosen, which are further based on the physiological state of an animal and the precursor metabolites available in the diet (Baldwin et al. 1980). Estimates of energy expenditure through metabolic

pathways can be determined at the molecular level (Rabkin and Blum, 1985) or at the physiological level for productive functions like milk secretion and growth (Baldwin et al. 1980). In this way, as information from numerous nutritional and biochemical studies becomes available it can be integrated into dynamic models which determine the energetic costs associated with metabolic functions.

The second approach, which is most often employed by nutritionists to assess energetic efficiencies of animals fed various feedstuffs (Lofgreen and Garrett, 1968 and Cammell et al. 1986), uses regression analysis of the changes in energy and nitrogen balances of individuals at different levels and quality of dietary intake. An empirical approach which partitions different levels of MEI into a maintenance requirement and the energy cost of protein and fat accretion has been used to do comparative studies of growth in different animal species. This technique has been used to investigate energetic costs in congenitally obese and normal rats (Pullar and Webster, 1977), ewe milk and pasture fed lambs (Rattray and Jagusch, 1977), pigs fed rations differing in level of energy and protein (Close et al. 1983) and different breeds of beef steers (Old and Garrett, 1985 and Old and Garrett, 1987). More recently, investigators have varied quality and amount of dietary protein to assess the metabolic response in growing pigs (Fuller et al. 1987) and energy intake restricted rats (Coyer et al. 1987). A summary of these studies gives reported estimated efficiencies of 0.45 to 0.50 for protein and 0.70 to 0.75 for fat deposition, in the simple-stomached animals such as rats and swine (Pullar and Webster, 1977; Fuller et al. 1987 and Coyer et al. 1987). Corresponding

ruminant values were more variable and lower, ranging from 0.10 to 0.40 for protein and 0.60 to 0.80 for fat deposition (Rattray and Jagusch, 1977; Old and Garrett, 1985 and Old and Garrett, 1987). The dynamics of energy and protein interactions continue to be of particular interest to animal nutritionists because positive returns to producers raising livestock for meat are usually associated with increased lean tissue accretion while fat deposition can represent a potential negative effect.

The integrated assessment of the energy requirements of animals being studied by empirical or even biochemical methods are often confounded by changes in metabolism of the individuals due to nutritional and physiological effects which are independent of the objective criteria under investigation (MacRae and Lobley, 1986 and Lobley, 1986). Extensive factors such as dietary level and quality, age, sex, species, environment and physiological state can alter interpretations of energy metabolism of animals (Koong et al. 1985). Unfortunately, previous reports using empirical approaches have invariably had to change these extensive factors to create the treatment differences used to make comparative estimates of the metabolic costs and corresponding energy efficiencies of animals. Changing the quality or quantity of diet or using apparently physiologically distinct animals are most often the criteria for comparative studies. However, within normal homeostatic regulation, the actual metabolism of the individuals being studied while on different extensive treatments is likely to be similar.

With the recent development of a number of $\beta_2$-adrenergic agonist growth promoting agents, there is the possibility of

actually manipulating the intensive aspects of metabolism such as ion transport and rate of protein turnover and thereby do a comparative study of treated individuals against control animals. In this way the treatments would not be affected by differences in extensive factors such as diet, developmental age and environmental conditions.

## B. CATECHOLAMINES AND BETA-RECEPTOR REPARTITIONING AGENTS

Cellular Messengers. Communication between cells and tissues within higher organisms is primarily mediated through the nervous and endocrine systems, both of which employ chemical messengers to convey biochemical and ultimately physiological responses. The nervous system sends discrete messages to specific target cell sites via a neurotransmitter receptor coupling at neural synapses whereas the endocrine system is more general, the hormones circulating throughout the body. Hormones secreted from endocrine glands into the blood stream selectively act on many cells, tissues and organs by coming in contact with specific receptors (Gorbman et al. 1983). Hormones which convey the chemical messages can act directly through a paracrine or autocrine mechanism but it is the endocrine function which sends the message throughout the body.

The catecholamines are chemical messenger derivatives of the amino acid tyrosine and are common to both the neural and endocrine communication networks. Norepinephrine and epinephrine act as transmitters for sympathetic nerves and the adrenal medulla, respectively, and generally initiate physiological responses which result in rapid energy expenditure in higher eukaryotes.

Epinephrine, and to a smaller extent norepinephrine, are synthesized and released into the blood stream by chromaffin cells in the adrenal medulla. Thus, they serve an endocrine function, producing a variety of responses such as contraction of the heart, dilation of the bronchial tract and increased contractile strength of skeletal muscle (Gorbman et al. 1983). The biological effects of catecholamines and the amplitude of response are mediated by the number and availability of surface membrane receptors at the targeted cells.

Classification of Catecholamine Receptors. Catecholamine receptors are ubiquitious, occurring in virtually all avian and mammalian tissues (Gorbman et al. 1983). Ahlquist (1948) first proposed the existence of two distinct types of adrenergic receptors which could elicit different physiological responses when bound by the catecholamines. Subsequently, based on pharmacological and radioligand binding studies, at least two classes of adrenergic cell receptors, alpha ($\alpha$) and beta ($\beta$), have been characterized which couple with the endogenous catecholamines as well as their pharmaceutical analogs (Fain and Garcia-Sainz, 1983). Further sub-classification of the beta and alpha receptors into $\beta_1$ and $\beta_2$ and $\alpha_1$ and $\alpha_2$, respectively, are based on different types and potencies of agonistic (activate the receptor) and antagonistic (block the receptor) analogs of epinephrine and norepinephrine (Crossland, 1980). The presence of at least four types of adrenergic receptors which are variably interspersed on different cellular membranes, makes it possible for catecholamines to mediate expression through either the neural or endocrine systems

of a multitude of different physiological responses at the cell, tissue, organ and species level. Table I.1 is a general summary indicating the origin of primary catecholamine chemical messengers, adrenergic receptor subtypes which are stimulated and the intracellular secondary messenger produced. These current adrenergic receptor subdivisions appear to be sufficient for categorizing the numerous drug analogs used for therapeutic purposes in medicine although it is likely that distinctive forms of the receptors may exist for various animal tissues (Timmerman, 1987).

Beta receptors and Adenylate cyclase. Lands et al. (1967) postulated the existence of two subtype $\beta$-adrenergic receptors. Both the $\beta_1$ and $\beta_2$ receptor subtypes have been shown by pharmacological studies to stimulate the membrane bound adenylate cyclase system, leading to an intracellular accumulation of the secondary messenger 3',5'-cyclic adenylate (cAMP) produced from adenosine 5'-triphosphate (ATP) (Stiles et al. 1984). The difference in $\beta$-receptor subtype classification is based on the relative affinity of epinephrine and norepinephrine for coupling to the respective receptor sites (Stiles et al. 1984). Norepinephrine which acts primarily as a neurotransmitter of the sympathetic nervous system is strongly active with $\beta_1$ sites (neural receptor) whereas epinephrine acting as a hormone predominately couples with $\beta_2$ sites (hormonal receptor). A number of hormones including glucagon and corticotropin also increase the intracellular cAMP concentration of their target tissues via activation of the adenylate cyclase system (Bramson et al. 1983).

Essential features for hormonal activation of the adenylate

cyclase system are reasonably well documented (Williams and Lefkowitz, 1978). The receptor-coupled sensitive adenylate cyclase moiety which responds to hormonal stimuli is composed of at least three component proteins: inhibitory and stimulatory receptor sites ($r_i$ and $r_s$), guanine nucleotide-binding regulatory units ($n_i$ and $n_s$) and the catalytic site. The three major components are capable of existing in both an active and inactive state and work in a concerted fashion. Initially, a hormonal agonist like epinephrine binds with the stimulatory receptor unit ($r_s$) at the cell membrane surface and through a low affinity coupling then forms a complex with the associated nucleotide regulatory unit ($n_i$). This intermediate complex is acted upon by guanosine 5'-triphosphate (GTP) and the agonist receptor component is uncoupled back to its low affinity form and the agonist is released (Brandt and Ross, 1986). The $n_i$-GTP protein complex in an activated state can interact with the catalytic unit to activate the production of cAMP. A GTPase moiety present in the $n_i$ protein cleaves GTP to GDP which destabilizes the complex and ultimately the system reverts back to a basal state, ready to be activated by the coupling of another agonist.

The cAMP end product of adenylate cyclase activation mediates it's effects at the molecular level through the regulation of cAMP dependent protein kinases. These kinases initiate the transfer of an α-phosphoryl group from $Mg^{++}$ATP to a serine or threonine residue at a specific site on substrate enzymes such as glycogen synthetase to either increase or decrease catalytic activity (Bramson et al. 1983). Ultimately, this phosphorylation of proteins

results in the turning on or off of metabolic pathways which result in the desired physiological responses.

Regulation of the adenylate cyclase system is the first point at which an outside stimuli's influence over cellular metabolism can be regulated. Mechanisms for regulation of $\beta$-adrenergic receptor function can be categorized into two broad classes (Stiles et al. 1984). Firstly, homologous desensitization denotes control of number and availability of receptors by the agonists which normally bind to them. This down regulation or the tendency for tissues to decrease biological response despite continuous agonistic stimulus does not appear to be controlled by the intracellular cAMP concentration (Sibley and Lefkowitz, 1985). In this homologous desensitization the receptors are uncoupled or translocated away from the regulatory and catalytic components. The sequestered receptors are then recycled or destroyed within the interior of the cell (Stiles et al. 1984 and Sibley and Lefkowitz, 1985).

Secondly, in heterologous regulation the control of adrenergic receptor concentration on cell membrane surfaces is modulated by hormones which do not bind with the receptor such as thyroxine and secondary messengers other than cAMP, like the prostaglandins. They inhibit or activate adenylate cyclase activity by a number of different mechanisms which have yet to be fully elucidated (Stiles et al. 1984). Nevertheless, heterologous desensitization of receptor function appears to occur by a phosphorylation of the adenylate cyclase moiety which uncouples or impairs the interaction between the nucleotide regulatory protein and the catalytic site (Stiles et al. 1984 and Sibley and Lefkowitz, 1985). The

cytoskeletal organization (Zor, 1983) and membrane lipid environment of cells (Houslay, 1985) have been implicated as having a role in the regulation of the membrane bound adenylate cyclase system. Predominance of a particular optical isomer or ionic species, which is dependent on pH, can also significantly alter the affinity that a $\beta$-agonist will have for $\beta$-receptors (Timmerman, 1987).

Alpha Receptors. Originally, alpha receptor subclasses were defined by their inhibiting effects on adenylate cyclase. However, $\alpha_2$ responses are associated with a drop in cAMP while $\alpha_1$ receptors appear to mediate an effect on the metabolic turnover of phosphatidylinositol which leads to an increase in the concentration of $Ca^{++}$ within the cell (McGrath, 1982). Unlike $\beta$ receptors the differentiation of the $\alpha$ subtypes is based on functional rather than anatomical criteria (Fain and Garcia-Sainz, 1980). These differences arise from the separate intracellular secondary messengers which are released upon receptor activation. An elevation of intracellular $Ca^{++}$ concentrations as a result of an increase in phosphatidylinositol turnover is mediated by $\alpha_1$-adrenergic receptors (Rasmussen and Barrett, 1984) while $\alpha_2$-receptors decrease concentrations of cAMP by inhibiting the adenylate cyclase system. The $\alpha_2$-adrenergic receptor is associated with the inhibitory guanine nucleotide regulatory protein and thereby the transduction of extracellular messages into the cell is down-regulated at the adenylate cyclase moiety (Cerione et al. 1985).

Beta-receptors and repartitioning agents. Beginning in the late 1970's researchers working in the laboratories of American Cyanamid

Company, Princeton, New Jersey, began a systematic search for pharmaceutic compounds which would partition nutrients towards greater muscle accretion and reduced fat deposition in the carcasses of domestic animals. Initial reports of this repartitioning effect were described in steers using a $\beta_2$-adrenergic receptor specific agonist given the generic name of clenbuterol (Ricks et al. 1984). A compound similar to clenbuterol, which is generically called cimaterol and is registered to the American Cyanamid Company (compound number AC or Cl 263,780) was also developed and successfully tested on mice (Asato et al. 1984). These orally active agents are phenylethanolamine derivatives which are structural congeners of epinephrine (Figure I.1). The ability of these $\beta$-agonists to change the homeostasis of animal metabolism to support a new physiological state has been termed homeorhesis (Ricks et al. 1984). A homeorhetic metabolism was first used to describe the physiological changes in lactating dairy cows (Bauman and Currie, 1980).

The activity of these agonists to bind $\beta$ rather than $\alpha$-receptor sites of the adenylate cyclase system (discussed earlier) is generally determined by a large substituent bound to the amino group of the ethanolamine side chain (Crossland, 1980). There are two $\alpha$ binding sites, one related to the ring structure and the more important amino nitrogen site which is hindered by the bulky nature of attached groups (Crossland, 1983). Specificity for binding affinity with $\beta_2$ rather than $\beta_1$-receptor sites depends on the substitutions made on the aromatic ring, particularly at position 3 which greatly hinders $\beta_1$ binding and therefore

increases $\beta_2$ coupling (Williams and Lefkowitz, 1978 and Timmerman, 1987). Compounds which do not have substitutions on position 3 of the phenylic ring usually act as antagonistic agents (Williams and Lefkowitz, 1978). Bulky substitutions on the amine of the ethanolamine side chain also increase $\beta_2$ selectivity (Timmerman, 1987). The specificity of repartitioning agents to bind selectively with $\beta_2$-receptors has been questioned, due to effects such as elevated tachycardia in lambs (Beermann et al. 1986b) and increased lipolysis in pigs (Mersmann et al. 1987), which are characteristic of $\beta_1$-receptor activation on the cellular membranes of heart and adipose tissues, respectively.

Physiological and metabolic effects of cimaterol. The $\beta$-agonist cimaterol is an orally active exogenous factor currently under investigation for the potential modification of growth patterns in domestic livestock. This $\beta$-agonist is an effective repartitioning agent, increasing muscle accretion and reducing fat deposition in the carcasses of rats (Sainz and Wolff, 1988), pigs (Mersmann et al. 1987), lambs (Beermann et al. 1986a), calves (Williams et al. 1987) and steers (Eisemann et al. 1988). The shift in the carcass parameters of these animals appears to be beyond the genetic predisposition for muscle hypertrophy under optimal nutritional strategies (Campbell, 1988). Improvements in carcass composition reported as greater muscle and less fat have been relatively similar between studies, the effects on daily gains and feeding efficiencies are more variable. Generally, the effects on body weight gain or feed conversion efficiency have not been consistent. For instance there have been positive reports in some

β-agonist treated lambs (Kim et al. 1987 and Hanrahan et al. 1987), while not in others (Hamby et al. 1986 and Beermann et al. 1986a). This variation in growth and feed performance is suprising considering that greater conversion of feed to weight gain should be correlated to balanced changes in the allometric growth pattern of cimaterol-treated animals, where a greater proportion of the carcass is lean tissue. However, improved feeding efficiency could be compromised by increases in energy expenditure due to the perturbation of metabolism in treated animals (Stock and Rothwell, 1986 and MacRae et al. 1988).

The metabolic and physiological mechanisms which are altered by β-agonist supplementation to animals are not clearly defined. There are a multitude of possibilities for divergent animal metabolism due to the variation of β-receptor subtypes within tissues of the same and different species (Timmerman, 1987). Furthermore, β-agonists are catecholamine-like compounds and there would be differences between animals for metabolism and excretion of these agents. The principle routes of disposal of catecholamines are by oxidative deamination and O-methylation catalyzed by monoamine oxidase and catechol-O-methyl transferase, respectively (Crossland, 1980). The products formed are further conjugated with glucuronates and sulphates at the hydroxyl groups of the phenyl ring, followed by excretion in the urine. Cimaterol and clenbuterol do not have hydroxylated phenyl rings (Figure I.1) therefore the only route of disposal of these agents is via monoamine oxidase catalyzed oxidative deamination, which differs in importance as a route of excretion among species. The

pharmacodynamic and pharmacokinetic variation in response to β-agonists, between species, makes a unified interpretation of the effects of these agents on animal metabolism difficult.

Ricks et al. (1984) have suggested that the changes in carcass composition of β-agonist treated animals may be a result of receptor-coupled stimulation of the adenylate cyclase system, initiating a cyclic-AMP mediated increase of lipolysis (Hu et al. 1987 and Hu et al. 1988) and glycogenolysis (Beermann et al. 1986b). Mobilization of lipid and glucose reserves as energy substrates causes anabolic sparing of muscle tissue through a reduction in protein degradation, with there being no apparent effect on protein synthesis (Reeds et al. 1986 and Bohorov et al. 1987). A corresponding alteration of skeletal muscle towards a hypertrophy of white and intermediate muscle fibers (fast twitch glycolytic and fast twitch oxidative glycolytic, respectively) appears to occur in rats (Maltin et al. 1987). On the other hand, hyperplasia of glycolytic muscle fibers occurs in lambs (Kim et al. 1987). At the molecular level, modulation of $Ca^{++}$-dependent muscle proteolytic enyzmes like the calpains, which initiate degradation of myofibrillar proteins starting at the Z-line, could be one of the mechanisms by which β-agonists might also directly reduce the rate of muscle breakdown (Higgins et al. 1988). The changes in the relative amounts of skeletal muscle and adipose tissue may be facilitated by an acute increase in blood flow to these tissues in animals treated with a β-agonist, however the chronic effect is a return to normal flow as well as a reduction in β-receptor density on the peripheral tissues (Rothwell et al.

1987).

Implication for cimaterol manipulation of livestock growth. From a practical standpoint, changing carcass composition, by itself is unlikely to be a sufficiently compelling reason for livestock producers to use $\beta$-agonists such as cimaterol. Current grading systems, together with an increasingly acerbated backlash from consumer interest groups towards pharmacological manipulation of farm animals, generally precludes any incentive for the use of exogenous growth promotants (Kempster, 1987). Consumption of products from animals treated with chemicals, therapeutic or otherwise, is often portrayed as a potential health risk to the public. Nonetheless, fulfilling burgeoning market demand for fast food products which contain red meats, as well as the continued economic viability of the livestock industry, may necessitate greater utilization of mature animals which are finished using growth manipulating agents. Indeed, $\beta$-agonists could offer producers economic opportunities by taking advantage of market price shifts, through a more regulated control of growth in their livestock. Clearly, definitive conclusions regarding the metabolic and energetic effects in treated animals are needed before the potential practical utility of $\beta$-agonists will be appreciated.

## C. OBJECTIVES

Study objectives. The objectives of the present studies were as follows:

(1) to assess the growth response and feeding efficiency of wether lambs fed cimaterol at low- and high-levels of dietary intake,

(2) to evaluate carcass characteristics and skeletal muscle, liver and heart weights and chemical composition in cimaterol-treated lambs,

(3) to determine total and $Na^+,K^+$-ATPase-dependent oxygen consumption in liver tissue biopsies of cimaterol-treated lambs,

(4) to determine physiological responses (heat production, heart rate and rectal temperature) and energy and nitrogen balances in lambs treated chronically with cimaterol and

(5) to determine the maintenance requirement and the energetic efficiencies of protein and fat accretion in cimaterol-treated lambs.

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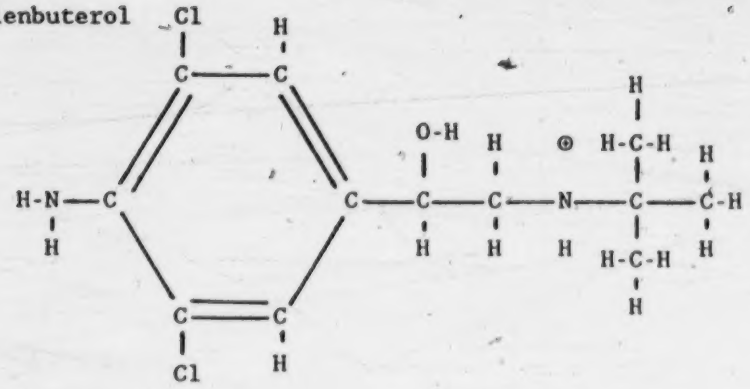
Table I.1. Site of release of primary neural and hormonal catecholamines, corresponding receptors that are bound and the intracellular secondary messenger mediating a metabolic response in mammalian cells*.

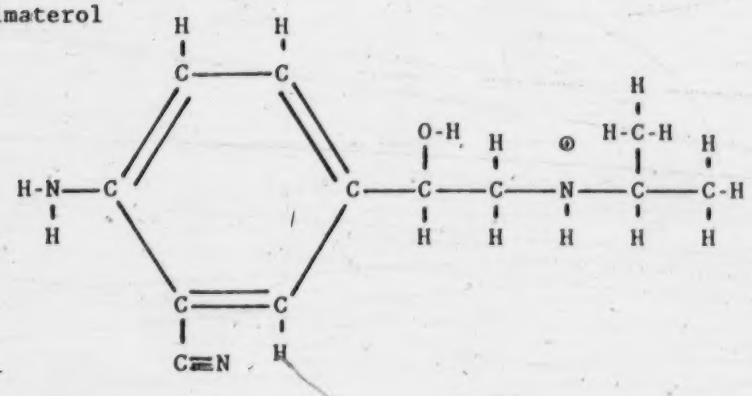
| Origin of release | Primary chemical transmitter | Receptor bound | Intracellular messenger |
|---|---|---|---|
| Sympathetic nerves | norepinephrine | $\alpha_1$ | elevated $Ca^{++}$ and phosphatidyl-inositol |
| Sympathetic nerves | norepinephrine | $\beta_1$ | elevated cAMP |
| Adrenal medulla | epinephrine | $\alpha_2$ | lower cAMP |
| Adrenal medulla | epinephrine | $\beta_2$ | elevated cAMP |

* Adapted from Fain, J.N. and Garcia-Sainz, J.A. (1983). Journal of Lipid Research 24, 945-965.

(a) Clenbuterol



(b) Cimaterol



(c) Epinephrine

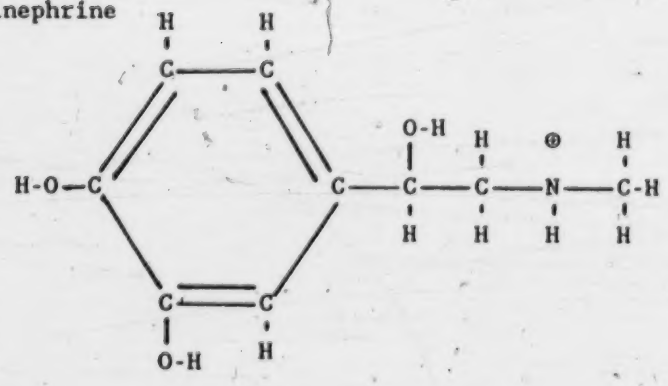


Fig. I.1. The chemical structures of the orally active phenylethanolamines (a) Clenbuterol, (±)-4-[amino-R-(t-butyl-amino) methyl-3,5-dichloro] benzyl alcohol and (b) Cimaterol, (±)-5-[1-hydroxy-2-(isopropylamino) ethyl] anthranilonitrile and the catecholamine (c) Epinephrine.

## II. GROWTH, BODY COMPOSITION AND OXYGEN CONSUMPTION OF LIVER TISSUE IN WETHER LAMBS SUPPLEMENTED WITH THE DIETARY β-AGONIST CIMATEROL*

### A. INTRODUCTION

Cimaterol (CL #263,780) a congener of the orally active phenylethanolamines, is currently being evaluated for it's β-adrenergic receptor selective ability to improve carcass characteristics of domestic livestock (Moser et al. 1986, Dalrymple and Inglé, 1987, Allen et al. 1987 and Hanrahan et al. 1987). This repartitioning agent apparently acts through a β-receptor coupled stimulation of the adenylate cyclase system, initiating intracellular cAMP mediated mobilization of triacylglycerol and possibly glycogen reserves as energy substrates (Beermann et al. 1986a). As a consequence β-agonists induce regulatory changes in the metabolism of treated animals, which suppress protein breakdown (Reeds et al. 1986 and Bohorov et al. 1987) causing a hypertrophy of skeletal muscle with less fat deposition in the carcass.

An increase in energy expenditure apparently accompanies the metabolic activity brought about by dietary β-agonists (MacRae et al. 1988 and Kim et al. 1989). Nevertheless, it is uncertain how

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* A version of this chapter has been submitted for publication. Caine, W.R. and Mathison, G.W. (1989). Br. J. Nutr.

shifts in the allometric growth pattern of β-agonist treated animals can also affect the components of metabolism associated with energy expenditure, particularly in non-carcass tissues. In cimaterol supplemented rats (Sainz and Wolff, 1988) and in calves treated with the β-agonist clenbuterol (Williams et al. 1987), alterations in the weight and chemical composition of the carcass appeared to be concomitant with changes in non-carcass tissues.

The size and metabolic rate of internal organs contribute both a substantial proportion and variation of the energy expenditure in animals (Webster, 1980 and Koong et al. 1985), although there is little information on the effects of β-agonists in these energetically intense tissues. The liver, which is the principal site of gluconeogenesis and is central to the clearance, synthesis and export of metabolites in the body, would be ideal for measurement of change in the energy metabolism of a specific tissue in β-agonist treated animals.

Notably, an estimated auxillary cost approaching 0.20 to 0.30 of maintenance energy expenditure is spent on the active transmembrane pumping of $Na^+$ and $K^+$ ions (Milligan and Summers, 1986). $Na^+,K^+$-ATPase (EC 3.6.1.3) activity is amplified in the tissues of animals under a variety of productive physiological conditions (Milligan and McBride, 1985) and is also an ancillary requirement for mitotic division of cells (Mummery et al. 1981). Active co-transport of metabolites by cytosolic $Na^+$ ion displacement with the extra/intra-cellular ionic gradient maintained by $Na^+,K^+$-ATPase is necessary for the uptake of amino acids and glucose by cells (Karasov and Diamond, 1983).

The objectives of this study were twofold. In conjunction with concurrent studies on energetic efficiencies (Chapter III) and whole body protein synthesis and degradation (Caine and Mathison, unpublished data), we determined growth response, carcass characteristics and chemical composition of various tissues in cimaterol supplemented wether lambs. Secondly, in view of the contribution of active transmembrane pumping of $Na^+$ and $K^+$ ions to total energy expenditure ( Milligan and Summers, 1986), we ascertained what effect the imposition of chronic dietary treatment with cimaterol had on in vitro total and $Na^+,K^+$-ATPase dependent oxygen consumption in hepatic tissue.

## B. EXPERIMENTAL

Design. At the commencement of the trial 15 crossbred Suffolk wether lambs (5 to 6 months of age) were matched by weight into three experimental groups of 5 animals each; control (33.0 kg, SD 3.7), cimaterol (34.3 kg, SD 6.9) and pair-treated (36.7 kg, SD 4.1). All lambs were initially fed at a low(L)-level which was subsequently followed by a high(H)-level period of dietary intake. The control group did not receive cimaterol at either the L- or H-level of dietary intake. Lambs in the cimaterol group received the $\beta$-agonist at the rate of 10 mg/kg of as-fed ration intake, over both the feeding periods. Pair-treated lambs received no cimaterol when fed at the L-level but received the $\beta$-agonist (10 mg cimaterol/kg of feed) during the H-level.

Animals, diet and management. The 15 lambs were held in individual metabolic crates and housed in two thermoneutral rooms,

(22.1 °C, SD 1.1) under continuous lighting. Prior to loading into the crates, lambs were closely shorn and given intramuscular injections (mg) of retinol 75, cholecalciferol 0.94, DL-α-tocopherol 25 and selenium 0.5. All animals were fed at a slightly above maintenance feeding level (55 g/metabolic body weight [$kg^{0.75}$] per d) good quality sun-cured alfalfa pellets for a 35 d adaptation period. Fresh water and cobalt-iodized salt were available free choice.

Formulation and analysis of the diets are contained in Table II.1. For the initial 25 ±2 d L-level period, lambs were fed alfalfa pellets and a barley premix at 46.8 and 6.6 $g/kg^{0.75}$ per d, respectively. The L-level ration was estimated to provide adequate dietary requirements for lambs at just slightly above a maintenance intake of energy and 15% crude protein using table values for the feed components (National Academy of Sciences, National Research Council, 1985). The barley premix contained a cimaterol carrier, which supplied the 10 mg/kg diet of the β-agonist to the treated animals or a corn meal equivalent for control wethers. Fresh water was available ad lib. For the second phase of the trial, lambs were switched to the H-level (equivalent to twice the L-level) dietary intake for an additional 50 ±1 days. Daily ration allocations were distributed equally over morning (08:30) and afternoon (16:30) feedings.

Lambs were weighed at the same time each week, 2-4 h after morning feeding, and dietary intakes were adjusted according to their weight changes on a biweekly basis. Once accustomed to the dietary level, an initial weight and average daily gain (ADG) was

calculated for each individual animal by regression of their weights against time. The ratio of live animal weight gain:dry matter (DM) intake was determined from the corresponding daily allocated ration.

Measurement of oxygen consumption in liver tissue biopsies. The procedure for obtaining liver biopsies and subsequently measuring oxygen consumption in these tissues is described by McBride and Milligan (1985). In brief, one day prior to slaughter lambs were suspended in a sling 2 to 4 h after morning feeding. Duplicate dorsal lobe liver tissue biopsies were obtained under local anaesthetic (5 ml 0.02 Lidocaine) and immediately transferred to glass cells containing 4 ml air-saturated Krebs-Hensleit buffer with 20 mM-Hepes and 10mM-D-glucose added. Respiration rates were then recorded at 37 °C in a polarographic $O_2$ electrode assembly (model #53, Yellow Springs Instrument Co, Ohio) with a Clark-type probe. Rates of oxygen consumption were apportioned between that required for $Na^+$, $K^+$ ion transport across membranes and other metabolic activity by inhibiting the $Na^+,K^+$-ATPase moiety with $10^{-4}$M ouabain. Oxygen consumption rates were expressed on a dry tissue weight basis.

Relative oxygen consumption contributed by the metabolic activity of the entire liver was estimated by assuming respiration rates of the biopsies were representative of the whole organ. Total dry weight of the livers were determined from analysis of samples collected the following day when lambs were sacrificed.

Slaughter Procedure. Lambs were systematically slaughtered at the conclusion of the H-level of dietary intake. To minimize the effects of stress on the day of sacrifice, animals were weighed

prior to receiving their morning ration and a polyvinylchloride (PVC) infusion line was attached to a jugular PVC catheter (1.01 mm ID, 1.67 mm OD) which had been fitted to each lamb the previous day. The infusion line was then extended through a porthole outside the room where the animals were housed. Two to four hours after morning feeding when lambs were resting they were deeply anaesthetized by a slow injection of 20-25 mg/kg body weight of thiopentone sodium through the infusion line and moved to a separate room, where they were killed by exsanguination. The liver and heart were immediately removed, flushed in ice-cold 0.9% saline solution and weighed. Warm whole carcasses were weighed and chilled for 24 h at 6 °C. Carcasses were subsequently reweighed and split transversely between the 11th and 12th ribs. Measurements were taken from both sides of the carcass for subcutaneous fat depth on the anterior surface of the 12th rib over the longissimus dorsi muscle, (in the fourth quadrant lateral from the dorsal spinuous process). Mean longissimus dorsi muscle areas were determined from traces of the 12th rib face on the left and right side of the carcass using a plainimeter. Psoas major, semitendinosus and gastrocnemius muscles were dissected out of the left side of the carcass and weighed.

Body tissue sampling. Whole body components were not available for chemical composition analyses, because of technical constraints caused by radioisotope residues present in tissues of the lambs as a result of a collateral study on protein turnover (Caine and Mathison, unpublished data). Alternatively, single samples of tissues (4-8 cm long, 2 cm wide) were taken from the same anatomical

site on each sheep. Medial transections of the left lateral lobe of the liver and right auricle and left ventricle of the heart were excised, sealed in 20 ml plastic scintillation vials and immediately stored frozen in liquid nitrogen, pending later analyses. Likewise, longitudinal pieces of backfat (lateral over the loin), perirenal fat (anterior to the kidney), psoas major, semitendinosus and gastrocnemius muscles, all from the right side of the carcass, were sampled and stored.

Chemical composition analyses. Tissue samples were thawed, weighed and diced into approximate 0.5 g pieces. Moisture content was determined by drying to a constant weight in a 100 °C forced draught oven. Crude fat was extracted into petroleum ether (boiling point 35-60 °C) and crude protein calculated from the nitrogen content of the residue samples using a standard macro-Kjeldahl procedure (Association of Official Analytical Chemists, 1984).

Statistical analysis. During the H-level, feeding efficiency and daily gain was determined only after animals were fully adjusted to consuming their entire allocated rations.

One of the pair-treated lambs died during the H-level period. Death was attributed to possible cardiac and respiratory failure, although the primary cause was not apparent. Weight changes and feed intake data collected from this animal were not used. All reported pooled standard errors are based on a harmonic mean of 4.62 observations per treatment.

Feed efficiencies and ADG were tested by a one way analysis of covariance procedure, using initial weight at the beginning of the low and high feeding levels as the respective covariate.

Similarly, the slaughter weight of lambs was used as a covariate for analysis of carcass characteristics. Tissue chemical composition and liver oxygen consumption data were analyzed by one way analysis of variance. Differences between treatment means were further analyzed using Duncan's new multiple-range test at 0.05 and 0.1 levels of significance (Steel and Torrie, 1980).

## C. RESULTS

Growth. Results for growth and feed performance are given in Table II.2. During the L-level period, ADG (g) for the cimaterol group (110) was improved ($P<0.05$) over the pair-treated lambs (29) and tended ($P<0.1$) to be greater than the control animals (41). There was a tendency ($P<0.1$) for live-wt gain:DM intake ratio to be increased for the cimaterol compared to control and pair-treated groups at the L-level. At the H-level, pair-treated lambs, which were now receiving cimaterol in the diet, showed a 0.26 improvement ($P<0.05$) in ADG compared to controls. The cimaterol group also had a corresponding 0.22 greater ($P<0.1$) ADG compared to control lambs at the H-level. There were no apparent improvements ($P>0.1$) in live-wt gain:DM intake ratio with the inclusion of cimaterol in the diet, at the H-level.

Oxygen uptake in liver tissue. Respiration rate indices for hepatic tissues and corresponding estimates for the whole liver are shown in Table II.3. Respiration rates (nmol $O_2$/mg dry tissue per min) were not different ($P>0.1$) for total (2.77, 2.26 and 2.71) and $Na^+,K^+$-ATPase-independent (1.94, 1.60 and 1.96) oxygen uptake in liver tissue biopsies from control, cimaterol and pair-treated

lambs, respectively. There was a trend ($P<0.1$) for $Na^+,K^+$-ATPase-dependent (ouabain sensitive) respiration to be reduced for the cimaterol (0.66) compared to the control (0.83) lambs, with the pair-treated (0.75) group showing an intermediate response. However, there was large variation within the pair-treated lambs (SE 0.09) which masked differences between the groups. Indeed, $Na^+,K^+$-ATPase-dependent respiration was significantly lower ($P=0.02$) for the cimaterol group when tested against only the control lambs.

Estimates of oxygen consumption (mmol $O_2$/h) for the entire liver, calculated from the dry weight of organs and respiration rates of biopsies, were not different ($P>0.1$). Although not significant ($P=0.24$) the $Na^+,K^+$-ATPase-dependent component of whole liver $O_2$ consumption was fractionally reduced by 0.21 and 0.08 for cimaterol and pair-treated lambs respectively, compared to controls.

Carcass characteristics. Carcass analyses values are given in Table II.4. The ratios of carcass to liveweight were greater ($P<0.05$) for both the cimaterol and pair-treated groups when compared to controls. Cimaterol and pair-treated lambs, respectively, showed fractional increases of 0.46 and 0.42 for gastrocnemius, 0.38 and 0.50 for psoas major and 0.27 and 0.26 for semitendinosus muscle weights over their control counterparts. Corresponding areas of the longissimus dorsi muscle were also improved 0.28 ($P<0.05$) and 0.15 ($P>0.1$).

Organ weights were not different ($P>0.1$) between treatments, although there was a reduction ($P<0.05$) in liver and heart ($P<0.1$)

weights in proportion to carcass in the treated animals (Table II.4). Conversely, combined muscle weights as a proportion of the carcass were increased ($P<0.05$) in the cimaterol group (18.4 g/kg carcass) compared to control lambs (16.0). The pair-treated group was intermediate (17.3).

Changes in backfat depth over the *longissimus dorsi* in response to cimaterol were more variable than the muscle parameters and appeared to be somewhat dependent on the time duration of administration of the $\beta$-agonist. A large range of variation for backfat depth in the pair-treated lambs (4.1 mm, SE 1.2) negated any significance ($P>0.1$) between the three groups. The backfat depth (mm) for the cimaterol lambs (3.2) was decreased ($P<0.001$, SE 0.3) when tested separately against only the control group (4.9).

*Tissue chemical composition*. The moisture, protein and fat content (g/kg wet weight) of the various tissue samples are given in Table II.5. Weights do not necessarily total 1000g/kg since as much as 0.08 and 0.01 of the wet weight of liver and muscle respectively, can be glycogen (White *et al*. 1964) and a further 0.01 of tissue weight is ash.

Dietary supplementation of cimaterol resulted in a variable response in chemical composition between the treatment tissue samples. Crude fat content of the samples representative of the three muscles were commonly reduced ($P<0.05$) for the cimaterol and pair-treated groups compared to control lambs. However, in comparison with the untreated controls, only the samples of the *psoas major* from the pair-treated lambs had a greater ($P<0.05$) proportion of weight as crude protein. Protein:fat ratios were

significantly ($P<0.05$) improved for cimaterol and pair-treated over control lambs, respectively, in the gastrocnemius (18.6, 15.0 and 7.3) psoas major (16.6, 13.1 and 5.0) and semitendinosus (8.5, 8.8 and 3.7) muscles. Both the cimaterol and pair-treated groups had a greater moisture content in the psoas major ($P<0.1$) and semitendinosus ($P<0.05$) samples when compared to controls.

The analyses of the chemical composition of backfat samples were not different ($P>0.1$) for the three treatments (Table II.5). Perirenal fat from the cimaterol treatment group contained ($P<0.1$) more moisture and less fat than control lambs. Corresponding pair-treated group values were intermediate. Likewise, the cimaterol lambs had more ($P<0.05$) protein in the liver and fat in the right auricle, with the pair-treated group having intermediate ($P>0.1$) values, compared to the controls. Protein:fat ratios were not different ($P>0.1$) between the three groups for liver, left ventricle and adipose tissues.

## D. DISCUSSION

Weight gains and characteristics of the carcasses in our treated lambs are in accord with reports on the effects of cimaterol in sheep (Kim et al. 1987 and Hanrahan et al. 1987). Interestingly, the shorter term administration of cimaterol to pair-treated lambs resulted in muscle hypertrophy similar to the cimaterol group which were fed the $\beta$-agonist for a longer time period. Although the cimaterol and pair-treated lambs received the $\beta$-agonist for a total of 75 and 50 d, respectively, both groups may have reached the optimal metabolic potential for lean tissue accretion.

Nevertheless, decreases in backfat depth of 0.53 for cimaterol and 0.28 for pair-treated lambs compared to the controls reflected reductions that appeared to be dependent on the duration of cimaterol administration. These lambs were approaching maturity where further lean tissue accretion in the carcass would occur at a decreased rate due to the inherent physiological limits of growth whereas, adipose reserves could have remained more susceptible to stimulation by the $\beta$-agonist. Hu et al. (1988) attributed a decrease in the carcass fat content of cimaterol-fed wether lambs to a lipolytic reduction in adipocyte size. Hence, backfat measurements in our cimaterol treated groups probably represent a variable ongoing mobilization of triacylglycerol reserves. Beta-adrenergic receptors are omnipresent in animal tissues, accordingly any divergence of responses is likely dependent on the stage of developmental growth and the $\beta$-receptor pharmacodynamics in the various tissues (Timmerman, 1987). In a study of cimaterol supplementation in young rapidly growing pigs in which there were no apparent effects on growth or carcass characteristics, the authors suggested that delineating the response of animals to treatment with $\beta$-agonists can be confounded by their nutritional and physiological status (Mersmann et al. 1987).

Previous studies to determine feed efficiencies in $\beta$-agonist treated animals have most often been at the ad lib. dietary level. In our study, where rations were strictly controlled, the data obtained from cimaterol supplemented growing lambs compared to physiologically similar controls is independent of any subtle confounding effects due to differences in dietary intake. A

positive growth response to dietary cimaterol supplementation resulted in a tendency ($P<0.1$) for 0.17 increase in body weight gain for the cimaterol group over the duration of the experiment. Pair-treated lambs attained only 0.04 greater weight gain compared to controls over the two dietary intake periods, although the gain was due to an apparent compensatory improvement ($P<0.05$) in growth performance during the H-level when they received the $\beta$-agonist.

Paradoxically, in contrast with a previous suggestion for an increased basal metabolic rate in cimaterol-treated lambs (Beermann et al. 1986a), we determined comparatively greater daily gains ($P<0.1$) and live-wt gain to DM intake ratios ($P<0.05$) at the near maintenance L-level (where minimal residual energy availability might be anticipated to have depressed growth) in cimaterol compared to control lambs. However, our experimental design did not allow for analyses of body composition after the L-level of intake and if lean tissue is accreted at the expense of fat deposition, weight gain can be increased while the actual energy content of the body remains unchanged or is reduced. Kim et al. (1989) were unable to find differences in the empty body weights between control and cimaterol-treated lambs fed at a maintenance level of intake, although there were changes ($P<0.05$) in carcass composition and weights. Gains in the carcasses of their maintenance-fed cimaterol lambs were increased for water, protein and ash and decreased for fat content ($P<0.01$). They also found that cimaterol significantly ($P<0.01$) elevated estimated fasting heat production and the metabolizable energy requirement for maintenance in these wethers.

Organ weights were not different ($P>0.1$) between our lamb

groups, although of interest was the disproportionately greater weight of the combined muscles ($P<0.05$) compared to liver ($P<0.05$) and heart ($P<0.1$) as a proportion of the carcass in the treated lambs. Kim et al. (1987) while reporting improved carcass quality and feed efficiency in cimaterol lambs, did not find differences from control individuals for body composition calculated from carcass density. Similarly, liver and heart weights in their cimaterol-treated lambs were not different ($P>0.05$) as a percentage of animal live weight. On the other hand, Hanrahan et al. (1987) did determine a decrease in the weights of liver, heart and internal fat deposits in entire male lambs supplemented with cimaterol. However, in corresponding cimaterol-treated wethers there were no differences from control animals for the weights of these same tissues (Hanrahan et al. 1987). Controlling intake of milk replacer to clenbuterol-treated calves, Williams et al. (1987) did not find differences in feed conversion or gain, but did determine a significant effect of the agent on the chemical composition of the whole body and of carcass and non-carcass tissues. In rats (Reeds et al. 1986) and calves (Williams et al. 1987) treated with clenbuterol, a shift in nitrogen deposition towards the carcass was apparently in part at the expense of non-carcass tissues.

Differences for the chemical composition of the tissue samples taken from the treated lambs in our study are in general agreement with the compositional changes in cimaterol-treated rats (Sainz and Wolff, 1988) and lambs (Hanrahan et al. 1987). Chemical analyses of muscle tissue samples of the cimaterol-treated lambs, indicated that fat was reduced ($P<0.05$) both as a fraction of wet weight or in

ratio to protein content. There was no discernible decrease in fat content for liver and heart tissues of treated animals, in fact our cimaterol lambs had proportionally ($P<0.05$) more fat in their right auricle tissues. A greater proportion of fat in heart tissue of the treated lambs is consistent with the shift in nitrogen deposition away from organ tissues in rats (Reeds et al. 1986) and calves (Williams et al. 1987).

Variation in weight (Koong et al. 1985) and metabolic intensity (Milligan and Summers, 1986) of internal organs in response to changing nutritional and physiological states is closely related to total energy expenditure. The internal organs account for 0.40-0.50 of total energy expenditure in animals (Webster, 1981), therefore any change in mass or metabolic activity of these tissues is likely to have significant consequences on whole animal energy metabolism. Baldwin et al. (1980) have even suggested that improved energetic efficiency may be imparted by animals accomplishing productive tasks without corresponding hypertrophy of metabolically intense tissues.

A substantial proportion of energy expenditure is contributed by the liver with it's metabolism in large measure dependent on the function of extra-hepatic tissues. Products of digestion entering the portal blood system from the intestine and metabolites in the hepatic arterial blood which have been released by extra-hepatic tissues, are removed by parenchymal cells of the liver to maintain relatively constant concentrations of circulating systemic blood metabolites. However, acute alteration in the concentrations of glucose, insulin and free fatty acids have been measured in the

plasma of cimaterol-treated lambs (Beermann et al. 1986b). These changes in metabolite concentrations of the blood could alter hepatic tissue metabolic activity. For instance, Eisemann et al. (1988) measuring arteriovenous differences across the hindquarters of 9 d chronically treated clenbuterol steers determined an increased release of L-lactate. If the same metabolic manifestations occur in cimaterol-treated sheep then it is likely that regeneration of L-lactate to glucose via the Cori cycle would increase the metabolic activity of hepatic tissue.

In our lambs, differences in liver metabolic intensity were not apparent, although it is interesting that there were non-significant ($P>0.1$) decreases of 0.18 and 0.02 in total liver $O_2$ consumption for the cimaterol and pair-treated groups from controls, respectively. An estimation of total energy expenditure contributed by the liver calculated from whole animal oxygen uptakes (measured earlier during the same time period as biopsies were obtained, in conjunction with an adjoining study [Chapter III]), were 0.04 for control, 0.03 for cimaterol and 0.04 for pair-treated lambs ($P>0.1$; SE 0.005). These liver to whole animal $O_2$ consumptions, as an indication of proportional energy expenditure are at some discord with previous estimates of 0.20-0.25 in domestic animals (Huntington and Reynolds, 1987), therefore making their absolute values questionable. A study of metabolic activity in hepatocytes of fed and fasted rats using a similar measurement system, but with incubation in a more complete suspension which contained a protein synthesis medium, obtained values approaching 16 nmol $O_2$/ mg dry wt per min (Burrin et al. 1988). This was an approximate twofold

increase in total $O_2$ consumption over rat studies with a minimal buffer system and some five times greater a respiration than we measured in our lambs. It would appear that our *in vitro* respiration measurements conducted using minimal media should not be used as a basis for a meaningful comparison of whole liver oxygen uptakes between the treatment groups.

Measurements of oxygen consumption from liver tissue of sheep have been variable depending on the physiological state of the animals under investigation. In hepatocytes isolated from fed versus starved sheep, total $O_2$ consumption (nmol $O_2$/ mg dry tissue per min) was not different ($P>0.05$), varying from 3.02 to 2.13, respectively (McBride and Milligan, 1985). Correlative respirations which ranged from 5.62 for infant lambs to 3.00 for mature sheep, were also not different ($P>0.05$). The 3.14 nmol $O_2$/ mg dry tissue per min reported in the same study for liver biopsies from non-lactating ewes being 0.13 greater than the respiration rate measured from our comparable control lambs.

Proportional estimates of total $O_2$ uptake accounted for by $Na^+,K^+$-ATPase-dependent respiration approached 0.30 for control and cimaterol-treated lambs. However, the $Na^+,K^+$-ATPase-dependent respiration may also have been overestimated because of potentially low values for total oxygen consumption of the biopsies. Nonetheless, there was a tendency ($P<0.1$) for a 0.2 decrease in respiration rate associated with hepatic $Na^+,K^+$ ion exchange in the cimaterol lambs (Table II.3). Like the decrease in backfat depth this effect was apparently conditional on the continuity of cimaterol

supplementation, since the reduction in $Na^+,K^+$-ATPase-dependent respiration for the pair-treated versus control lambs was only 0.1. $Na^+,K^+$-ATPase respiration in liver tissue of sheep accounts for a substantial proportion of energy expenditure which apparently changes depending on the physiological state of animals (Milligan and McBride, 1985). Therefore, a diminished $Na^+,K^+$-ATPase-dependent respiration in the cimaterol compared to control lambs suggests there could be a departure from physiological normalcy which is dependent on the length of $\beta$-agonist treatment.

We conclude that dietary treatment with cimaterol shifted the allometric growth pattern of our treated lambs. Gains in carcass lean tissues were preferentially at the expense of proportional increases in the organ tissues, with a collateral reduction of fat content in muscles. The tendency for reductions ($P<0.1$) in $Na^+,K^+$-ATPase respiration of liver tissue in treated lambs, suggests that regulatory aspects of metabolism may be altered by cimaterol. Ongoing treatment with the $\beta$-agonist could suppress the relative contribution made by the active $Na^+,K^+$ ion transport system to basal metabolism and overall energy expenditure. The potential effects of dietary $\beta$-agonists offers a regulated opportunity to contrast the various components of energy metabolism in animals, which warrants further study.

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Table II.1. Composition and analyses of experimental diets fed to wether lambs at low and high levels of intake

| | Control* | Cimaterol* |
|---|---|---|
| Ingredients (g/kg air dry basis). | | |
| Sun-cured alfalfa pellets | 876 | 876 |
| Ground barley | 94 | 94 |
| Calcium phosphate† | 14.5 | 14.5 |
| Vitamin premix‡ | 0.5 | 0.5 |
| Trace mineralized salt§ | 5 | 5 |
| Corn meal | 10 | - |
| Cimaterol (1g/kg carrier) | - | 10 |
| Analysis | | |
| Dry matter (DM)(g/kg) | 919.0 | 920.0 |
| Nitrogen (g/kg DM) | 24.5 | 24.4 |
| Organic matter (g/kg DM) | 888.7 | 885.0 |
| Gross energy (MJ/kg DM) | 18.1 | 18.1 |

* Pair-treated lambs were fed as controls during the low-level and the same as the cimaterol group at the higher intake period.

† Supplying (g/kg diet): phosphorous 3.0 and calcium 2.6.

‡ Supplying (mg/kg diet): retinol 1.5, cholecalciferol 0.0125 and DL-α-tocopherol 5.0.

§ Supplying (mg/kg diet): sodium chloride 4800, zinc 20, iron 8, manganese 6, copper 1.65, iodine 0.35 and cobalt 0.2.

Table II.2. Dry matter (DM) intake, average daily gain (ADG) and live-weight gain:dietary DM intake ratio of wether lambs fed at low- and high-levels of intake

(Means adjusted by covariance for differences in initial live weight at the beginning of both feeding levels together with pooled standard errors)

| | Treatment | | | |
|---|---|---|---|---|
| | Control | Cimaterol | Pair-treated | SE* |
| No. of animals | 5 | 5 | 4 | |
| Regressed initial wt (kg) | 32.8 | 34.2 | 37.5 | 2.6 |
| Low-level | | | | |
| Cimaterol ($\mu g/kg^{0.75}$ per d) | - | 529 | - | 6 |
| Days on test | 21 | 21 | 21 | 0 |
| DM ($g/kg^{0.75}$ per d) | 47 | 48 | 49 | 1 |
| ADG (g) | $41^{ab}$ | $110^{a}$ | $29^{b}$ | 25 |
| Live-wt gain:dietary DM (g/g) | $0.06^{ab}$ | $0.16^{a}$ | $0.04^{b}$ | 0.04 |
| High-level | | | | |
| Cimaterol ($\mu g/kg^{0.75}$ per d) | - | 976 | 988 | 16 |
| Days on test† | 45 | 45 | 42 | 2 |
| DM ($g/kg^{0.75}$ per d) | 89 | 93 | 94 | 3 |
| ADG (g) | $266^{a}$ | $325^{ab}$ | $336^{b}$ | 21 |
| Live-wt gain:dietary DM (g/g) | 0.17 | 0.19 | 0.18 | 0.01 |

a,b Within rows, means with different superscript are significantly different ($P<0.05$).

* One of the pair-treated animals died during the experiment, therefore pooled SE is based on a harmonic mean of 4.62 observations per treatment.

† Measurements taken only after lambs were adjusted to the higher level of dietary intake.

Table II.3. Total oxygen consumption, percentage inhibition by ouabain, $Na^+,K^+$-ATPase-dependent and -independent respiration for hepatic tissue biopsies and corresponding estimates of whole liver $O_2$ uptake in lambs.

(Treatment means with a pooled standard error)

| | Treatment* | | | |
|---|---|---|---|---|
| | Control | Cimaterol | Pair-treated | SE† |
| No. of animals | 5 | 5 | 4 | |
| Days on cimaterol | 0 | 74 | 48 | 0.3 |
| Whole liver dry wt (g) | 235 | 231 | 246 | 16 |
| Liver tissue $O_2$ consumption (nmol $O_2$/mg dry tissue per min) | | | | |
| Total | 2.77 | 2.26 | 2.71 | 0.25 |
| $Na^+,K^+$-ATPase: | | | | |
| -dependent‡ | 0.83[c] | 0.66[d] | 0.75[cd] | 0.06 |
| -independent | 1.94 | 1.60 | 1.96 | 0.23 |
| Fractional inhibition of respiration by ouabain | 0.30 | 0.29 | 0.28 | 0.03 |
| Estimated total liver $O_2$ consumption (mmol/h) | | | | |
| Total | 39.1 | 31.7 | 39.5 | 4.4 |
| $Na^+,K^+$-ATPase: | | | | |
| -dependent‡ | 11.7 | 9.2 | 10.8 | 1.1 |
| -independent | 27.4 | 22.6 | 28.7 | 3.8 |

[c,d] Within rows, means with different superscripts are significantly different ($P<0.1$).

* Cimaterol lambs were treated with the $\beta$-agonist for 25 ±2 d at a near maintenance level of dietary intake followed by 49 ±1 d at a higher level. Pair-treated lambs were fed cimaterol at the higher level of dietary intake only.

† One of the pair-treated animals died during the experiment, pooled SE is based on a harmonic mean of 4.62 observations per treatment.

‡ $Na^+,K^+$-ATPase-dependent respiration determined as the ouabain ($10^{-4}$ M) sensitive component.

Table II.4. Effect of cimaterol on carcass characteristics and liver, heart and muscle weights
(Means adjusted by covariance for differences in slaughter weights with pooled standard errors)

| | Treatment | | | |
|---|---|---|---|---|
| | Control | Cimaterol | Pair-treated† | SE* |
| No. of animals | 5 | 5 | 4 | |
| Slaughter wt (kg) | 48.4[c] | 52.4[d] | 53.8[d] | 3.1 |
| Cold carcass wt (kg) | 21.4[c] | 25.3[cd] | 27.3[d] | 0.6 |
| Carcass wt:live-wt | 0.442[a] | 0.481[b] | 0.506[b] | 0.01 |
| Backfat depth (mm) | 4.9 | 3.2 | 4.1 | 0.7 |
| Longissimus d. area ($cm^2$) | 14.6[a] | 18.7[b] | 16.8[ab] | 0.9 |
| Muscle wt (g) | | | | |
| Gastrocnemius | 103[a] | 150[b] | 146[b] | 9 |
| Psoas major | 103[a] | 142[b] | 154[b] | 5 |
| Semitendinosus | 135[a] | 172[b] | 170[ab] | 7 |
| Organ wt (g) | | | | |
| Liver | 848 | 823 | 843 | 41 |
| Heart | 218 | 211 | 231 | 12 |
| Proportional wt (g tissue/kg carcass) | | | | |
| Combined muscles | 16.0[a] | 18.4[b] | 17.3[ab] | 0.5 |
| Liver | 39.7[a] | 32.9[b] | 31.0[b] | 1.7 |
| Heart | 10.3[c] | 8.5[d] | 8.5[d] | 0.5 |

[a,b] Within rows, means with different superscripts are significantly different ($P<0.05$).

[c,d] Within rows, means with different superscripts are significantly different ($P<0.1$).

* One of the pair-treated animals died during the experiment, pooled SE is based on a harmonic mean of 4.62 observations per treatment.

† Pair-treated lambs were fed as controls over a 25 ±2 d low-level dietary period and then received cimaterol (10 mg/kg diet) during a 50 ±1 d high-level of feed intake.

Table II.5. Moisture (M), crude protein (P) and fat (F) as a ratio of the wet weight of tissue samples taken from control, cimaterol and pair-treated lambs
(Treatment means with a pooled standard error)

| | Treatment | | | |
|---|---|---|---|---|
| | Control | Cimaterol | Pair-treated† | SE* |
| Liver | | | | |
| M | 0.687 | 0.674 | 0.675 | 0.006 |
| P | 0.190[a] | 0.211[b] | 0.200[ab] | 0.005 |
| F | 0.13 | 0.13 | 0.11 | 0.02 |
| P:F | 18.1 | 17.1 | 18.2 | 3.3 |
| Right auricle | | | | |
| M | 0.740[c] | 0.662[d] | 0.707[cd] | 0.022 |
| P | 0.113 | 0.111 | 0.115 | 0.008 |
| F | 0.099[a] | 0.185[b] | 0.161[ab] | 0.026 |
| P:F | 1.5[c] | 0.6[d] | 0.9[cd] | 0.3 |
| Left ventricle | | | | |
| M | 0.734 | 0.765 | 0.773 | 0.017 |
| P | 0.130 | 0.138 | 0.155 | 0.010 |
| F | 0.054 | 0.038 | 0.036 | 0.011 |
| P:F | 4.7 | 4.8 | 5.8 | 2.1 |
| Gastrocnemius muscle | | | | |
| M | 0.737 | 0.747 | 0.735 | 0.005 |
| P | 0.211 | 0.237 | 0.232 | 0.014 |
| F | 0.029[a] | 0.013[b] | 0.017[b] | 0.002 |
| P:F | 7.3[a] | 18.6[b] | 15.0[b] | 1.8 |
| Psoas major muscle | | | | |
| M | 0.746[c] | 0.759[d] | 0.761[d] | 0.005 |
| P | 0.183[a] | 0.208[ab] | 0.229[b] | 0.011 |
| F | 0.038[a] | 0.014[b] | 0.019[b] | 0.003 |
| P:F | 5.0[a] | 16.6[b] | 13.1[b] | 1.9 |
| Semitendinosus muscle | | | | |
| M | 0.746[a] | 0.765[b] | 0.766[b] | 0.006 |
| P | 0.180 | 0.186 | 0.182 | 0.007 |
| F | 0.051[a] | 0.022[b] | 0.024[b] | 0.005 |
| P:F | 3.7[a] | 8.5[b] | 8.8[b] | 1.1 |
| Backfat | | | | |
| M | 0.171 | 0.210 | 0.204 | 0.027 |
| P | 0.035 | 0.042 | 0.033 | 0.006 |
| F | 0.743 | 0.721 | 0.688 | 0.026 |
| P:F | 0.05 | 0.06 | 0.05 | 0.01 |
| Perirenal fat | | | | |
| M | 0.146[c] | 0.254[d] | 0.165[cd] | 0.039 |
| P | 0.015 | 0.014 | 0.014 | 0.002 |
| F | 0.823[c] | 0.708[d] | 0.799[cd] | 0.04 |
| P:F | 0.02 | 0.02 | 0.02 | 0.0 |

[a,b] Within rows, means with different superscripts are significantly different ($P<0.05$).

[c,d] Within rows, means with different superscripts are significantly different ($P<0.1$).

* One of the pair-treated animals died during the experiment, pooled SE is based on a harmonic mean of 4.62 observations per treatment.

† Pair-treated lambs were fed as controls over a 25 ±2 d low-level dietary period and then received cimaterol (10 mg/kg diet) during a 50 ±1 d high-level of feed intake.

## III. Influence of the β-agonist cimaterol on energetic efficiencies of growing lambs*

### A. INTRODUCTION

Cimaterol (CL #263,780), an orally active pharmaceutic analogue of the catecholamines, has recently been reported to improve the protein:fat ratio in carcasses of pigs (Jones et al. 1985 and Moser et al. 1986), lambs (Kim et al. 1987 and Chapter II), poultry (Dalrymple and Ingle, 1987) and cattle (Allen et al. 1987 and Boucque' et al. 1987). The effect of β-agonists is mediated by homeorhetic changes in the metabolism of treated animals such that skeletal muscle deposition is enhanced while triacylglycerol reserves are apparently catabolized (Beermann et al. 1986a). Other direct and indirect mechanisms may also be associated with this β-agonist induced repartitioning effect (Buttery and Dawson, 1987).

The energy requirements of growing animals have been determined by the separation of energy expenditure into a maintenance requirement and costs of tissue deposition using regression analyses of data collected over a number of dietary levels of intake (Close et al. 1983 and Old and Garrett, 1985). The demarcation of energy expenditure into maintenance and productive functions does not

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describe the dynamic variations of metabolism, particularly when the maintenance component changes with the nutritional and physiological state of the animals studied (Koong et al. 1985, Ferrell et al. 1986 and Milligan and Summers, 1986). Nevertheless, nutritionists find the technique useful for evaluating the interrelationship between basal metabolism and growth in livestock (Garrett, 1980).

Assessment of the energy requirements of animals is often confounded by changes in factors such as dietary quality, age, sex, species, environment and physiological state, which are the criteria used by researchers for comparative studies (Koong et al. 1985). Use of a $\beta$-agonist with the potential to alter the actual metabolic activity associated with accretion of protein and fat tissue could provide further insight into the the energy metabolism of animals. Unfortunately, quantitative information concerning the effect of $\beta$-agonists on energy expenditure to fulfill maintenance requirements and the energetic costs associated with protein and fat accretion is limited.

On a weight basis if more lean and less adipose tissue is being deposited in the carcasses of $\beta$-agonist treated animals this should reduce the incremental requirement of dietary energy intake for each unit of growth. In a recent study of cimaterol-supplemented lambs Kim et al. (1989) determined increases of 0.19 in maintenance requirement, 0.14 in fasting heat production and a decrease of 0.13 in daily energy gain over controls animals. Similarly, 0.11 greater energy expenditure and 0.55 less energy retention were reported in cimaterol-treated rats (Sainz and Wolff, 1988).

Currently there are no reports of the energy cost of protein deposition in cimaterol-treated animals. Protein synthesis is estimated to account for a substantial proportion of the energy expenditure in animals (Lobley, 1986). However, hypertrophy of muscle tissue in rats (Reeds et al. 1986), calves (Williams et al. 1987 and sheep (MacRae et al. 1988) by treatment with the β-agonist clenbuterol was a result of a decrease in protein degradation with no change in the rate of synthesis. Therefore, a dichotomy exists in that energy expenditure is apparently elevated in β-agonist treated animals, although there is plausibly a lower energy cost of lean muscle accretion due to a reduction in protein breakdown.

We studied the energy requirements for maintenance and energetic efficiencies associated with protein and fat tissue assimilation in growing lambs by manipulating the composition of their growth using cimaterol (Chapter II). Furthermore, measurements of heart rate and rectal temperature were taken in conjunction with heat production measurements to elucidate possible long-term physiological changes associated with cimaterol treatment of lambs.

## B. MATERIALS AND METHODS

Design. The experimental design consisted of the three treatment groups (control, cimaterol and pair-treated) each with five lambs sequentially fed at a low (L)- followed by high (H)-level of dietary intake. Diets were formulated to provide the β-agonist at 10 mg/kg of dietary intake as-fed basis to the cimaterol treatment group over both the L- and H-levels.

Pair-treated lambs were fed as controls (the agent not included in the diet) for the L-level and then received the $\beta$-agonist at 10 mg cimaterol/kg of dietary intake during the H-level. As the pair-treated group were a treatment equivalent to the controls at the L-level, measurements for pair-treated lambs were taken only at the H-level.

Animals and management. Fifteen growing crossbred Suffolk wether lambs with an initial mean weight of 31.4 (SD 5.2) kg were housed in individual metabolic crates in two continuously illuminated thermoneutral rooms 22.1 (SD 1.1) °C. Lambs were fed 55 g/kg metabolic live weight ($kg^{0.75}$) per d of good quality sun-cured alfalfa pellets, with a cobalt-iodized salt block and fresh water available free choice, for a 35 d adaptation period. Lambs were then randomly assigned into control, cimaterol or pair-treated groups in a restricted manner to achieve similar mean live weights for each of the treatments.

Measurements were made at the two levels of dietary intake. For the initial L-level of intake the ration consisted of alfalfa pellets (46.8 $g/kg^{0.75}$ per d) and a barley premix (6.6 $g/kg^{0.75}$ per d) which contained the cimaterol carrier or a corn meal equivalent for controls. Equal proportions of the daily ration were fed at 08:30 and 16:30. This diet allocation was estimated to provide approximately 1.1 times the metabolizable energy intake (MEI) required for maintenance of the lambs. During the second experimental period at the H-level, the same alfalfa pellets and fresh barley premix were fed at the rate of 93.6 $g/kg^{0.75}$ and 13.2 $g/kg^{0.75}$ per d respectively to deliver an estimated 2.2 times

maintenance dietary intake. Animals were weighed weekly and their intake adjusted to weight changes on alternate weeks. Formulation and analyses of the diets were reported previously (Chapter II).

Experimental measurements. Stepwise initiation of treatments were conducted at both feeding levels so that all measurements for the lambs could be made after the same time duration. A 7 d collection of faeces and urine was carried out after 14 d adaptation to the diet. On day 22 of treatment, 24 h determinations of heat production and methane losses were made by indirect calorimetry. Lambs had previously been habituated to spending extended periods of time with their head and neck enclosed in a ventilated respiration hood (Webster and Hicks, 1968) from which respired $O_2$, $CO_2$ and $CH_4$ gases were measured with the open-circuit calorimetry system described by Young et al. (1975). The apparatus was modified by using in-line wet and dry bulb thermometer measurements to determine flow of dry air thereby eliminating the cold trap. Further, the addition of a series of in-line two-way solenoid air switching valves (Asco 1" orifice, Ascolectric Ltd., Ontario) controlled electrically by a four circuit recycling timer (Chron Trol® model #CT-4, Lindburg Enterprises, California), allowed for simultaneous measurements of gas exchange in the last 10 min of alternate 15 min samplings from two lambs in hoods. Data output from the analyzers was stored using a DT100 Data Logger (Data Electronics, Aust. Pty. Ltd.). The whole system was calibrated using an Fe-burner apparatus (Young et al. 1984). Heat production (HP) was calculated using the equation of Brouwer (1965).

Concurrent with respiration measurements, a continuous record of

rectal temperature (RT) was collected from a 12 cm polyethylene covered temperature probe inserted into the rectum of each lamb . Heart rates (HR) were monitored at the beginning of each hour for a 5 min period using pin type electrodes which were placed at the foreflanks and over the loin of each lamb and connected to a Beckman physiological recorder (model R-612, Electronic Instruments Division, Illinois) controlled by the recycling timer.

Analytical procedure. Urine was collected in 20 ml 6N HCL containing 1 g/L mercuric chloride. Faeces were trapped on a mesh suspended below the metal grid floor of animal crates. Aliquots of daily urine and faecal excretions were pooled for the 7 d collection period and stored at -20 °C until analyzed. Dry matter (DM) was determined for feed and faeces by drying to a constant weight in a 105 °C forced-draught oven. Crude protein was calculated from nitrogen (N) content of feed, faeces and urine using the standard macro-Kjeldahl procedure (Association of Official Analytical Chemists 1984). Corresponding gross energy (GE) contents were measured in automatically controlled bomb calorimeters (Parr adiabatic model 1241, Parr Instrument Comp., Illinois).

Calculations to partition MEI. Energy retention (RE), determined from energy balance data, as protein ($RE_p$) was calculated from N retention X 6.25 using a value of 23.6 KJ/g of protein. Fat ($RE_f$), having an energy equivalent of 39.3 KJ/g, was estimated by difference between RE and $RE_p$ (Agricultural Research Council (ARC) 1980). Efficiencies for retention of energy in tissue for the control and cimaterol groups were determined from the difference in RE at the two ME intakes scaled by $kg^{0.75}$ for each

lamb. Similarly, utilization of MEI for maintenance was calculated from individual lamb HP and MEI data using the semilog-linear approach of Lofgreen and Garrett (1968). Fasting heat production (FHP) was also estimated as the Y-intercept from the same regression of log HP on MEI.

Multiple regressions through the origin were used to estimate the energy costs of protein and fat deposition. Calculated maintenance requirements for each lamb were subtracted from MEI at both levels of energy intake to give metabolizable energy available for production ($ME_{prd}$), which was then partitioned following the model of Old and Garrett (1985) where: $ME_{prd} = bRE_p + cRE_f$. The corresponding b and c regression coefficients in this equation represent the incremental energy requirements to deposit protein and fat respectively.

Statistics. The data were analyzed as a split-block design. Because the pair-treated group was measured only at the H-level, least-squares analysis of variance of unequal subclass numbers (Harvey 1960) was computed. Sources of variation were treatment (T=3), animals within treatment (n=5,5,4), feeding level (L=2) and their interactions. Treatment was tested against the animals within treatment term. Feeding levels and treatment by feeding level interactions were tested using the residual error term. Where there were trends ($P<0.1$) for differences due to treatments or the interactions, means within the feeding levels were further tested for simple effects (Steel and Torrie, 1980). Analysis of covariance was used to compute and test homogeneity of the treatment regression coefficients to partition $ME_{prd}$ between $RE_p$ and $RE_f$.

Differences in energetic efficiencies between treatments were also tested for significance by Student's t test (Steel and Torrie, 1980).

## C. RESULTS

Energy and nitrogen balances. Dry matter, N and energy intakes were not different ($P>0.1$) between treatments, but were significantly ($P=0.001$) changed by dietary intake level (Table III.1). No differences ($P>0.1$) were found in the apparent digestibilities of DM and energy due to $\beta$-agonist supplementation, although decreases ($P<0.01$) of approximately 0.05 were associated with the increase in feed intake. Apparent digestibility of N was also not different ($P>0.05$) between treatments at the L-level but was improved ($P<0.05$) for the cimaterol compared to control and pair-treated lambs at the H-level. Losses of urinary N were increased ($P=0.001$) by about 0.55 at the H-level for all lambs but there were no changes ($P=0.86$) due to cimaterol treatment. The proportions of digestible energy (DE) lost as methane (KJ/KJ) were 0.13 for control and 0.15 for cimaterol lambs at the L-level ($P<0.05$). Methane/DE at the H-level was greater by 0.16 ($P<0.05$) for the cimaterol compared to pair-treated lambs.

Heat production and energy retention. Hourly HP values measured before and after morning feeding at 08:30 on the first day of treatment at the L-level, are shown in Figure III.1. Heat production was not different ($P=0.65$) prior to the morning ration. Although variable, there was a trend ($P=0.09$) for a thermogenic

increase of 0.15 in the first hour post-feeding for cimaterol compared to control lambs, when HP prior to feeding was used as a covariant. However, this apparent rise in HP for the treated group was subsequently not different ($P>0.1$) from control lambs 2 hours post-feeding.

MEI, HP and energy retentions at the L- and H-levels are presented in Table III.2. Heat production and MEI were significantly ($P=0.001$) changed by feeding level but not altered ($P>0.1$) by cimaterol treatment. Actual means of HP (MJ/d) for the control and cimaterol groups were 6.5 and 6.8 at the L-level compared with 10.2, 10.2 and 10.4 for control, cimaterol and pair-treated lambs, respectively at the H-level of intake ($P=0.57$; SEM 0.68).

Cimaterol increased ($P<0.05$) N retention and gross efficiency of N utilization at the H-level, although the effect was not apparent ($P>0.05$) at the L-level. Retention of energy as protein and fat was increased ($P=0.001$) at the H-level in comparison to the L-level (Table III.2). There was also a corresponding increase ($P<0.05$) in energy retention as protein ($KJ/kg^{0.75}$ per d) in cimaterol (124.1) and pair-treated (113.7) lambs compared to controls (98.9) at the higher feed intake. At the L-level of intake, when residual energy available for growth was minimal, positive protein deposition apparently caused a concomitant negative $RE_f$ ($KJ/kg^{0.75}$ per d) of -2.8 and -25.9 for control and cimaterol lambs, respectively.

Hourly heart rate, rectal temperature and heat production. The effect of cimaterol supplementation on hourly heart rate, rectal temperature and heat production for the L- and H-levels of intake is

set out in Figures III.2, III.3 and III.4 respectively. Physiological and thermogenic measurements were more variable at the L- compared to the H-level for both control and cimaterol lambs. In the statistical analysis of the data, 24 h measurement periods were subdivided into timed intervals. Times were as follows: 04:00 to 07:00 as a basal period, 09:00 to 11:00 post-morning feeding, 12:00 to 14:00 intervening midday and 17:00 to 19:00 post-evening feeding. Daily means as well as the time period effects for HR, RT and HP are presented in Table III.3. Time of day had a significant effect ($P<0.001$) on HR and HP but not on RT ($P>0.1$). Physiological changes due to cimaterol supplementation or interactive treatment (T) X period (P) effects were not apparent ($P>0.1$). Rectal temperatures did tend ($P=0.08$) to be elevated for cimaterol compared to control and pair-treated lambs at the H-level, although the increase was not large.

Partitioning of metabolizable energy intake. The partition of MEI into energy costs associated with maintenance and tissue deposition, as well as the partial efficiencies for $RE_p$ and $RE_f$, using regression analysis are given in Table III.4. Estimates of MEI required for the maintenance of control and cimaterol lambs were not different ($P=0.69$), at 438 and 458 $KJ/kg^{0.75}$ per d respectively. Corresponding FHP of 322 and 348 KJ/kg 0.75 per d were also not significant ($P=0.58$; SEM 32). The resultant multiple regression equations for protein and fat deposition were:

Control: $ME_{prd}$=2.54(SE 0.51) $RE_p$+1.13(SE 0.31) $RE_f$; R 0.99, P<0.001, n 5

Cimaterol: $ME_{prd}$=1.65(SE 0.32) $RE_p$+1.30(SE 0.21) $RE_f$; R 0.99, P<0.001, n 5

The coefficients for $RE_p$ and $RE_f$ between treatments were not significantly different (P=0.27 and P=0.41, respectively). Congruous efficiencies for energy deposition of 0.67 for cimaterol lambs compared to 0.57 for controls were also not different (P=0.36, Table III.4).

## C. DISCUSSION

Our experimental design, including measurements of pair-treated lambs at the H-level, allowed us to account for any potential carry over effects associated with previous treatment and physiological state on energy expenditure for the treated group.

Nitrogen balances and methane losses. Nitrogen retentions were significantly (P<0.05) greater for both the cimaterol and pair-treated groups compared to controls at the H-level, yet N digestibilities were improved (P<0.05) only for the cimaterol lambs (Table III.1). Conversely, Kim et al. (1989) attributed increases in N retention for six cimaterol lambs fed at 0.8 of ad lib. intake to reduced losses in urine and not to any changes in N digestibilities, based on a 14 d collection period after 7 days adaptation. In the present study, urinary N losses were reduced, although not significantly (P=0.86) at the H-level by 0.09 and 0.06 for the cimaterol and pair-treated compared to control lambs respectively. Overall improvement (P<0.05) in energy retention as

protein, for the two β-agonist treated groups is consistent with the positive effects of cimaterol on the gain in skeletal muscle weights (Chapter II).

Proportional losses of DE as methane energy were increased ($P<0.05$) by 0.15 in the cimaterol lambs at the L-level, when nutrient availability would be at a minimum, however methane losses were not different ($P>0.05$) from controls at the H-level. This apparent change in the proportion of DE lost as methane between the two dietary levels in the cimaterol compared to control group, indicates that supplementation of β-agonists could alter the dynamics of fermentation within the reticulo-rumen. In general α-mediated stimulation accentuates reticulo-ruminal contractions while β-effects at least partly attenuate tension and amplitude of spontaneous contractions of rumen smooth muscle in ruminants (van Miert, 1987). Thus use of a predominantly β-specific agent could result in a suppression of rumen motility and possibly duration of contractions leading to a slower passage rate of nutrients out of the reticulo-rumen into the lower gastro-intestinal tract. Greater energy losses as methane at the L-level would be consistent with a delay in passage rate out of the rumen (Okine et al. 1989) in β-agonist treated ruminants.

Decreased volatile fatty acid (VFA) concentrations in the rumen liquor of steers treated with 500 mg/head per d of clenbuterol was thought to be due to an inhibition of rumen motility and feed intake, with a resultant depression of the fermentative process (Ricks et al. 1984). In a study by Boucque' et al. (1987), a 0.05 increase in rumen pH and a greater proportion of VFA as acetate and

propionate were measured in cimaterol-treated wethers. These researchers also determined a 0.02 decrease in crude fiber digestibility which obfuscates the apparent reasoning for reduced rumen motility as the cause of increased methane losses or changes in the pattern of VFA production.

Heat production. The lack of a thermogenic effect observed in our chronic cimaterol-treated lambs is in agreement with the results of other studies. Rikhardsson et al. (1988) measured an initial 0.1 to 0.2 elevation in HP from days 1 to 4, which was not apparent by 8 to 10 d in lambs treated with cimaterol,. Rothwell and Stock (1988) also did not find differences in energy expenditure between control (22 d), adrenalectomized (14 d), clenbuterol-treated (22 d) and clenbuterol-treated adrenalectomized (14 d) rats using comparative slaughter analysis. On the other hand increases in HP due to $\beta$-agonists have been reported, usually in rodents (Stock and Rothwell, 1986). Energy expenditures were increased by twice daily subcutaneous injections of clenbuterol for 16 d and fenoterol for 19 d in male rats (Emery et al. 1984), clenbuterol supplementation for 105 d in milk replacer to Friesian bull calves (Williams et al. 1987) and by including cimaterol for 12 d in the diet of rats (Sainz and Wolff, 1988). Heat productions were also greater for wether lambs treated with dietary clenbuterol during a 20 d period (MacRae et al. 1988) and with cimaterol for a 90 d feeding trial (Kim et al. 1989).

With the exception of two of these studies (MacRae et al. 1988 and Rikhardsson et al. 1988), assessments of energy expenditure in $\beta$-agonist treated animals were interpreted from comparative

slaughter trials, which evaluate the cummulative energy and nitrogen balances of animals. Thus, over a time duration, they would not differentiate between acute or chronic changes in response by animals. MacRae et al. (1988) measured a variable HP response directly by gas exchange calorimetry in lambs treated with clenbuterol. They noted a marked initial increase in thermogenic response to the $\beta$-agonist during days 1 to 5 which, with the duration of treatment, became less apparent from 6 to 20 days. The period of clenbuterol treatment in their trial lasted 20 d while measurements in our lambs were initiated after 21 d of supplementation. Indeed, in our cimaterol-treated lambs there was an increase of 0.15 in HP over the controls one hour after morning feeding, on the first day of treatment at the L-level (Figure III.1). The thermogenic response to the $\beta$-agonist was not apparent within 2 hours after feeding. Furthermore, cimaterol-treated lambs in the Rikhardsson et al. (1988) study also did not have an increased HP after 8 to 10 days.

Activation of $\beta_1$-adrenoreceptors in brown adipose tissue (BAT) is a source of non-shivering and diet-induced thermogenesis (Stock and Rothwell, 1986). Since rats and young animals have shown a $\beta$-agonist induced increase in HP while our lambs which were approaching maturity did not suggests that thermogenic responses to cimaterol, at least in part, could also be dependent on the presence of BAT.

Physiological responses such as increased tachycardia have been inferred as the source of elevated HP in $\beta$-agonist treated animals. However, the pattern of hourly heart rate, HP and rectal

temperature in our cimaterol-treated lambs compared to controls were not different (Table III.3). This is in contradiction to an acute rise in heart rates of about 250 beats/min and elevated rectal temperature to 41 °C demonstrated within 4 h treatment with clenbuterol for lambs nourished wholly by intragastric infusion (Herbert et al. 1985). Later studies though, have shown heart rates which transiently increase by 0.80 to 1.2 return to normal after a 12 to 24 h period of adaptation to cimaterol (Beermann et al. 1986b). In clenbuterol-treated steers, heart rates increased to 150 beats/min on day 1 of treatment but returned to a more normal 110 beats/min by 9 days (Eisemann et al. 1988). Our data seems to support the concept of transient adjustment in response to cimaterol treatment. Nevertheless, erratic shifts in heart rates for the cimaterol-treated lambs during periods of feeding, did indicate a lingering ability for the $\beta$-agonist to elicit at least a moderate increase in physiological response when provoked by environmental stimuli (Figure III.2).

Maintenance requirement and energy costs of tissue deposition. The estimates of MEI required for maintenance ($KJ/kg^{0.75}$ per d) calculated by the regression method in our study were similar for the control (438) and cimaterol (458) lambs ($P=0.69$, Table III.4). Corresponding MEI for maintenance reported by Rikhardsson et al. (1988) of 313.8 and 305.4 were also not different between control and cimaterol lambs, although their estimates were approximately 0.3 lower than ours. Kim et al. (1989) reported significantly different values for maintenance energy expenditure (KJ $MEI/kg^{0.75}$ per d) of 387.9 and 461.1 respectively between control and cimaterol lambs

fed for 90 d at maintenance and ad lib. levels of dietary intake. Similarly, FHP did not appear to be different between our groups whereas the estimates of Kim et al. (1989) were significantly ($P<0.01$) elevated by 0.13 for cimaterol compared to control lambs.

Energy costs, determined from regression coefficients, associated with the deposition of protein were not different ($P<0.05$) between control and cimaterol lambs (Table III.4). Although, the efficiency of protein gain of 1.65 KJ MEI/KJ for the cimaterol-treated group compared with that of 2.54 for the controls does accord with lower energy cost of lean tissue deposition. The estimate for the control lambs is similar to an incremental average value of 2.3 KJ MEI/KJ for accretion of protein which has been reported for monogastrics (Pullar and Webster, 1977). Ostensibly, the similarity of energy cost of protein deposition determined for our control group when fed a good quality diet and monogastrics, further indicates that the biochemical transformations involved with growth are not energetically dissimilar for ruminants. Reports for the MEI requirements associated with protein growth in young ruminants are variable. Upper limit estimates (KJ MEI/KJ) determined by regression analysis for protein deposition of 6.02 in early weaned grazing lambs (Rattray and Jagusch, 1977) and 9.28 in fattening steers (Old and Garrett, 1985) have been reported. On the other hand, a theoretical minimal efficiency of 1.19 KJ MEI/KJ, independent of any protein turnover, can be estimated from the ascribed 4.5 KJ MEI/g protein synthesized, assuming 5 mols ATP are required for the formation of one peptide bond (Millward et al. 1976).

Many previous researchers (Pullar and Webster, 1977; Close et al. 1983 and Coyer et al. 1987) have discussed the limitations of regression analysis of energy expenditure. The high degree of apparent collinearity which exists between coefficients, confounds parallel estimates of energy expenditure for protein and fat deposition. The derived incremental values therefore are not necessarily independent estimators of the energy requirements associated with the biochemical realities which are brought about by changes in energy intake or allometric growth manipulations. An alternative empirical approach to determine maintenance requirements and energy cost of protein accretion was employed, which is similar to the technique used by Close et al. (1983) and later Coyer et al. (1987) and Fuller et al. (1987). A fixed coefficient value of 1.32 KJ MEI/KJ for $RE_f$ was adopted from stoichiometric estimates of MEI required for fat accretion (from Baldwin, 1968; Milligan, 1971 and Lobley, 1986). Where lambs were in a negative fat balance it was assumed this reflected mobilization of adipose reserves, in which case a value of 1 KJ MEI/KJ was used as the coefficient for $RE_f$, since in the conversion of triacylglycerol to glycerol and free fatty acids there is an estimated loss of only 0.01 of the energy content of the triacylglycerols (Millward et al. 1976). MEI minus the energy cost of fat deposition, as a residual MEI ($ME_{res}$), was then used to simultaneously calculate maintenance and protein assimilation requirements. Calculated values of maintenance requirements and cost of protein deposition as mathematical expressions were:

Control: $ME_{res}$-2.91(SE 0.59) $RE_p$+ 378 (SE 48), n 5

Cimaterol: $ME_{res}$-1.98(SE 0.28) $RE_p$+ 425 (SE 26), n 5

The maintenance requirements (KJ MEI/$kg^{0.75}$ per d) for control (378) and cimaterol (425) lambs were again not different ($P$=0.42), although the mean for the treated lambs was 0.12 greater than that for the controls. These corresponding maintenance requirements were 0.07 and 0.14 respectively lower in comparison with our regression estimates (Table III.4), but there were no significant differences ($P$>0.1) in the values determined by either procedure. Calculated costs of protein deposition were also not different ($P$=0.19) between treatments. Partial efficiencies for protein assimilation of 0.34 for control and 0.50 for cimaterol lambs were lower by 0.13 and 0.17 respectively, when the cost of fat accretion is fixed compared to the regression approach. That the maintenance requirements were less and protein deposition costs greater with a fixed fat coefficient suggests that some of the energy expenditure which results from metabolic activities associated with lean tissue assimilation may be masked by inclusion as part of maintenance.

The estimated cost of 1.13 KJ MEI/KJ for fat accretion in the controls was inordinately low, although it is similar to a theoretical efficiency of 1.15 KJ MEI/KJ for fat production where carbohydrate or protein intermediates are used as substrate precursors (Millward et al. 1976). The $\beta$-agonist apparently did not alter energy costs of fat deposition, the 1.30 KJ MEI/KJ coefficient the same as the empirical value of 1.32 for fat

synthesis; assuming acetate as the precursor with reducing equivalents ($NADPH_2$) supplied from propionate (from Baldwin, 1968; Milligan, 1971). Corresponding costs of fat deposition are equally variable ranging from the biologically nonsensical coefficient of 0.87 in lambs (Rattray and Jagusch, 1977) to 1.72 KJ MEI/KJ for fat gain in steers (Old and Garrett, 1985).

Accordingly, there are certain limitations in attributing physiological and biochemical significance to the mathematical partition of MEI between its components, however an amalgamation of our data does suggest interesting shifts in energy assimilation and the metabolic processes which might bring about these changes between cimaterol and control lambs. Apportionment of energy costs between the main components of energy expenditure are consistent with the supposed metabolic changes in $\beta$-agonist treated animals, where cimaterol lambs might be depositing more protein with a greater efficiency than their control counterparts since the incremental MEI required for protein accretion in the treated lambs approached the theoretical energy costs of oneway synthesis without any turnover. A reduction in protein degradation has been suggested as a possible metabolic action for $\beta$-agonists (Reeds et al. 1986, Williams et al. 1987 and MacRae et al. 1988).

In conclusion, we were unable to attribute any significant differences between the cimaterol-treated and control lambs for total HP or the energy expenditure associated with maintenance and tissue deposition. Nevertheless, the lack of elevated HP and rectal temperature or any prolonged tachycardial effects is consistent with a reduction in $Na^+,K^+$-ATPase respiration in liver tissue of

cimaterol-treated compared to control lambs (Chapter II). Although not significant, the energy costs associated with protein deposition were reduced by 0.32 with this decrease in energy expenditure counterbalanced by an apparent increase of approximately 0.12 in the maintenance requirement, determined when the energy cost of fat accretion was assumed constant.

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Table III.1. Daily intakes, losses of nitrogen (N) in urine and methane production as proportions of digestible energy (DE) and apparent digestibilities of dry matter (DM), N and energy for lambs on Treatment (control, cimaterol and pair-treated) by Level of Dietary intake (low and high)

| Dietary Level | Low | | High | | | | Level of significance‡ | | |
|---|---|---|---|---|---|---|---|---|---|
| | Control | Cimaterol | Control | Cimaterol | Pair-treated* | SEM† | L | T | LXT |
| No. of animals | 5 | 5 | 5 | 5 | 4 | | | | |
| Live weight (kg) | 33.5 | 36.4 | 41.6 | 43.4 | 44.5 | 0.6 | 0.001 | 0.76 | 0.42 |
| Intake: | | | | | | | | | |
| DM (g/d) | 671.4 | 700.2 | 1446.4 | 1486.9 | 1541.3 | 38.7 | 0.001 | 0.74 | 0.88 |
| N (g/d) | 18.0 | 18.6 | 38.6 | 39.4 | 40.9 | 1.0 | 0.001 | 0.79 | 0.92 |
| Energy (MJ/d) | 12.2 | 12.7 | 26.1 | 26.8 | 27.8 | 0.7 | 0.001 | 0.75 | 0.89 |
| Losses: | | | | | | | | | |
| Urinary N (g/d) | 8.9 | 8.9 | 14.5 | 13.2 | 13.7 | 0.6 | 0.001 | 0.86 | 0.31 |
| Urine energy (MJ/d) | 0.8 | 0.8 | 1.2 | 1.1 | 1.3 | 0.01 | 0.001 | 0.62 | 0.30 |
| Methane/DE (KJ/KJ) | 0.13[a] | 0.15[b] | 0.10[fg] | 0.11[g] | 0.09[f] | 0.004 | 0.001 | 0.09 | 0.34 |
| Apparent digestibilities | | | | | | | | | |
| DM | 0.67 | 0.67 | 0.63 | 0.64 | 0.63 | 0.01 | 0.004 | 0.84 | 0.21 |
| N | 0.71 | 0.71 | 0.66[f] | 0.70[g] | 0.66[f] | 0.01 | 0.011 | 0.25 | 0.06 |
| Energy | 0.69 | 0.68 | 0.64 | 0.66 | 0.65 | 0.01 | 0.008 | 0.96 | 0.18 |

a,b Means with different superscripts were different at the Low dietary level ($P<0.05$).

f,g Means with different superscripts were different at the High dietary level ($P<0.05$).

* Pair-treated lambs were fed as controls for the low-level and the same as the cimaterol group during the higher intake period. Observations for the pair-treated group taken at the high-level only.

† Standard error of the interaction means (SEM), df=8 and based on a harmonic mean of 4.62 observations per treatment.

‡ Two-way analysis of variance where: L, Level of Diet; T, Treatment and L X T, Level X Treatment interaction.

Table III.2. Metabolizable energy intake (MEI), heat production (HP), retentions of nitrogen and energy (RE) as well as gross efficiencies in lambs on Treatment (control, cimaterol and pair-treated) by Level of Dietary intake (low and high)

(Values expressed as KJ/kg$^{0.75}$ per d unless otherwise stated)

| Dietary Level | Low | | High | | | | Level of significance‡ | | |
|---|---|---|---|---|---|---|---|---|---|
| | Control | Cimaterol | Control | Cimaterol | Pair-treated* | SEM† | L | T | LXT |
| MEI | 505.1 | 477.5 | 898.1 | 923.6 | 936.0 | 23.4 | 0.001 | 0.80 | 0.27 |
| HP | 466.9 | 462.1 | 627.9 | 604.5 | 605.5 | 17.9 | 0.001 | 0.81 | 0.60 |
| Nitrogen retained (g/d) | 3.9 | 4.3 | 11.0$^{f}$ | 14.4$^{g}$ | 13.4$^{g}$ | 0.6 | 0.001 | 0.30 | 0.03 |
| RE | 38.1 | 15.4 | 270.1 | 319.1 | 330.5 | 30.1 | 0.001 | 0.73 | 0.25 |
| Energy retained as: | | | | | | | | | |
| Protein§ ($RE_p$) | 41.0 | 41.3 | 98.9$^{f}$ | 124.1$^{g}$ | 113.7$^{g}$ | 3.9 | 0.001 | 0.34 | 0.01 |
| Fat ($RE_f$) | -2.8 | -25.9 | 171.2 | 195.0 | 216.8 | 29.4 | 0.001 | 0.74 | 0.43 |
| Gross efficiencies (retained:intake) | | | | | | | | | |
| Nitrogen | 0.217 | 0.225 | 0.287$^{f}$ | 0.362$^{g}$ | 0.328$^{g}$ | 0.01 | 0.001 | 0.38 | 0.02 |
| Energy | 0.044 | 0.018 | 0.169 | 0.200 | 0.205 | 0.02 | 0.001 | 0.83 | 0.23 |

f,g Means with different superscripts were different at the High dietary level ($P<0.05$)

* Pair-treated lambs fed as controls during the low-level and the same as the cimaterol group at the high-level.

† Standard error of the interaction means (SEM), df=8 and based on a harmonic mean of 4.62 observations per treatment.

‡ Two-way analysis of variance where: L, Level of Diet; T, Treatment and L X T, Level X Treatment interaction.

§ $RE_p$ calculated as 148.1 KJ times nitrogen retained with $RE_f$ calculated as the difference; RE-$RE_p$

Table III.3. Effect of cimaterol supplementation at low- and high-levels of dietary intake on heart rate, heat production and rectal temperature in wether lambs on Treatment by Period of Time (pre- and post-feedings)

| | Time periods* (h) | | | | | | Level of‡ significance | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0400-0700 | 0900-1100 | 1200-1400 | 1700-1900 | Daily | SEM† | T | P | TXP |
| Low-level | | | | | | | | | |
| Heart Rate (beats/min) | | | | | | | | | |
| Control | 84 | 102 | 101 | 95 | 92 | 14 | 0.14 | 0.002 | 0.48 |
| Cimaterol | 92 | 125 | 107 | 117 | 107 | | | | |
| Heat Production ($KJ/kg^{0.75}$ per h) | | | | | | | | | |
| Control | 17.1 | 22.0 | 21.1 | 20.7 | 19.5 | 2.0 | 0.78 | 0.001 | 0.95 |
| Cimaterol | 16.3 | 22.6 | 20.2 | 20.2 | 19.3 | | | | |
| Rectal Temperature (°C) | | | | | | | | | |
| Control | 39.0 | 39.6 | 39.9 | 38.8 | 39.1 | 0.4 | 0.49 | 0.097 | 0.49 |
| Cimaterol | 38.7 | 39.2 | 39.3 | 39.3 | 38.9 | | | | |
| High-level | | | | | | | | | |
| Heart Rate (beats/min) | | | | | | | | | |
| Control | 106 | 140 | 121 | 129 | 120 | 16 | 0.31 | 0.001 | 0.53 |
| Cimaterol | 120 | 149 | 142 | 132 | 133 | | | | |
| Pair-treated | 105 | 128 | 117 | 127 | 121 | | | | |
| Heat Production ($KJ/kg^{0.75}$ per h) | | | | | | | | | |
| Control | 23.2 | 28.1 | 27.6 | 29.6 | 26.2 | 2.5 | 0.61 | 0.001 | 0.18 |
| Cimaterol | 23.2 | 26.5 | 25.3 | 28.1 | 25.2 | | | | |
| Pair-treated | 24.1 | 24.5 | 24.8 | 29.1 | 25.2 | | | | |
| Rectal Temperature (°C) | | | | | | | | | |
| Control | 39.9 | 40.1 | 39.9 | 40.0 | 39.9 | 0.6 | 0.08 | 0.520 | 0.50 |
| Cimaterol | 40.4 | 40.3 | 40.5 | 40.3 | 40.4 | | | | |
| Pair-treated | 39.2 | 39.4 | 39.5 | 39.8 | 39.4 | | | | |

* Time periods based on a 24 h clock; 0400-0700, resting pre-morning feeding; 0900-1100, post-receiving morning ration at 08:30; 1200-1400, interlude; 1700-1900, post-receiving afternoon ration at 16:30 and daily means.

† SEM for interaction means. One of the pair-treated lambs died during the experiment, SEM at the H-level are based on a harmonic mean of 4.62 observations per treatment.

‡ Probability level of difference where T, Treatment with cimaterol; P, Period of Time and T X P, interactive effect.

Table III.4. Estimated energy cost of maintenance, protein and fat deposition for control and cimaterol lambs

| | Control | SE | Cimaterol | SE | Probability of difference |
|---|---|---|---|---|---|
| Energy associated with: | | | | | |
| Maintenance ($KJ/kg^{0.75}$ per d) | 437.7 | 45.9 | 458.3 | 19.8 | 0.69 |
| Tissue Deposition (KJ/g)* of | | | | | |
| Protein | 59.9 | 12.0 | 38.9 | 7.6 | 0.27 |
| Fat | 44.4 | 12.2 | 51.1 | 8.3 | 0.41 |
| Efficiency of MEI use for energy gain | 0.57 | 0.09 | 0.67 | 0.05 | 0.36 |
| Partial efficiencies for energy gain† as | | | | | |
| Protein | 0.39 | | 0.60 | | |
| Fat | 0.88 | | 0.77 | | |

* Values calculated from regression coefficients assuming 23.6 KJ/g protein and 39.3 KJ/g fat (ARC, 1980).

† Inverses of regression coefficients.

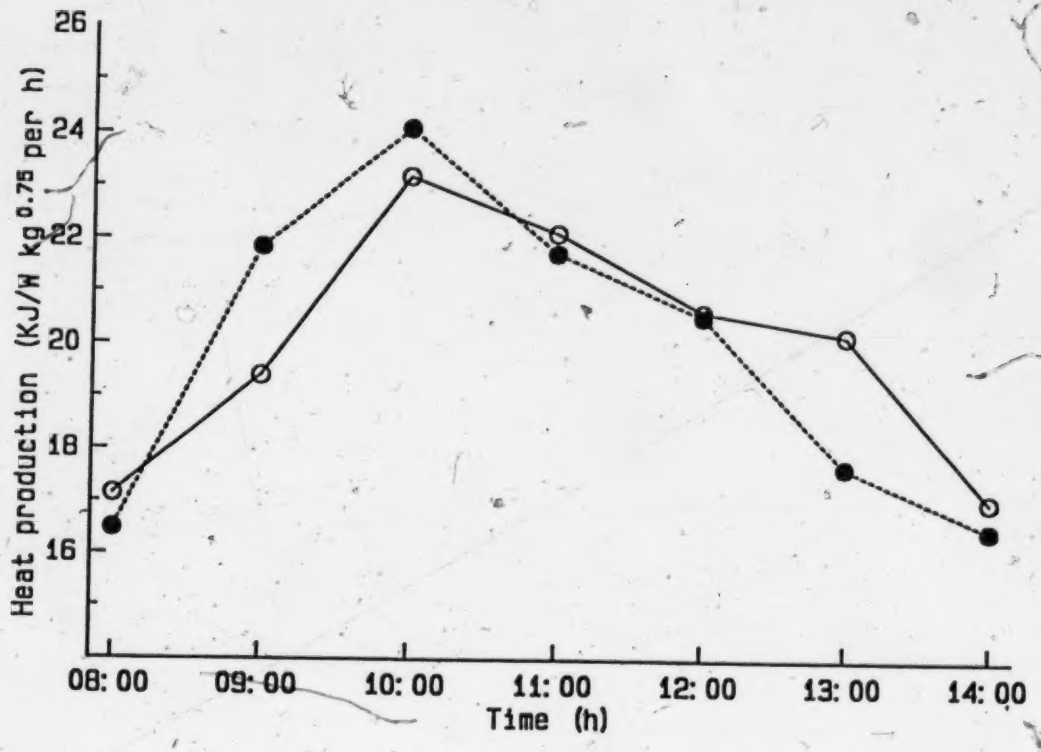
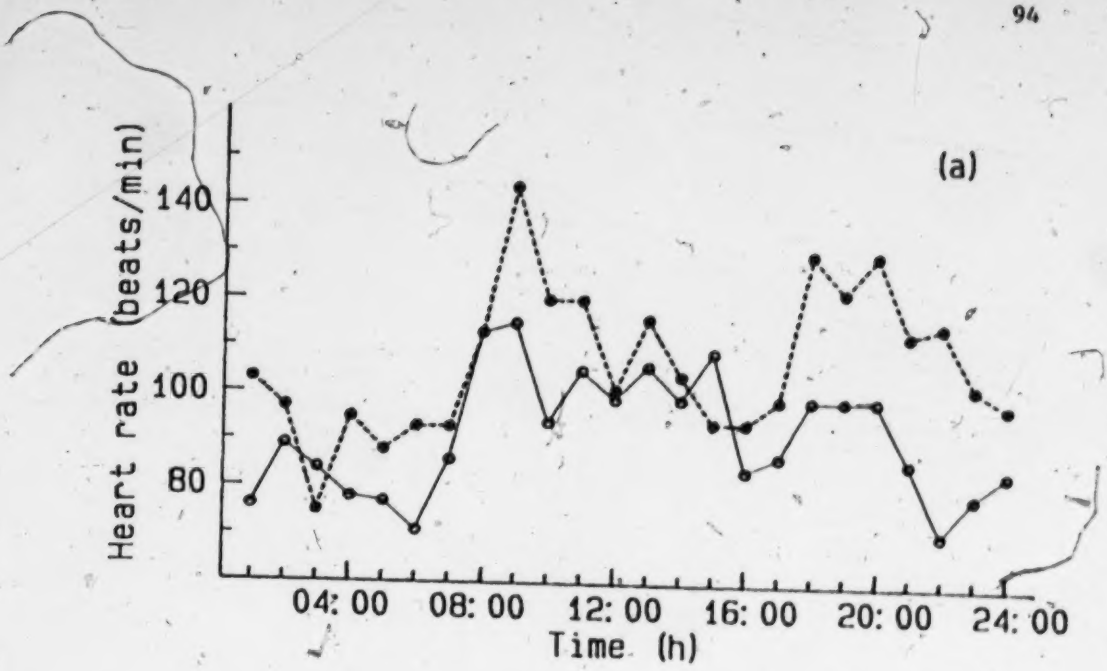



Fig. III.1. Hourly heat production of control (o——o) and cimaterol-supplemented (•– – –•) lambs, before and after feeding the 08:30 morning ration on day 1 of initiating treatment at the low-level of dietary intake. Results are expressed as means with five observations (SEM 1.1).



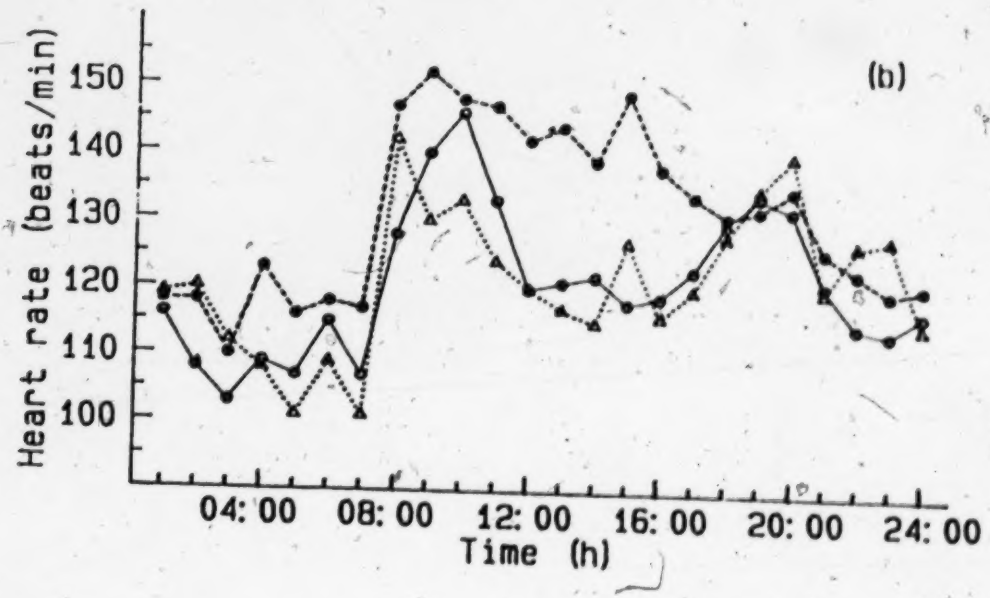
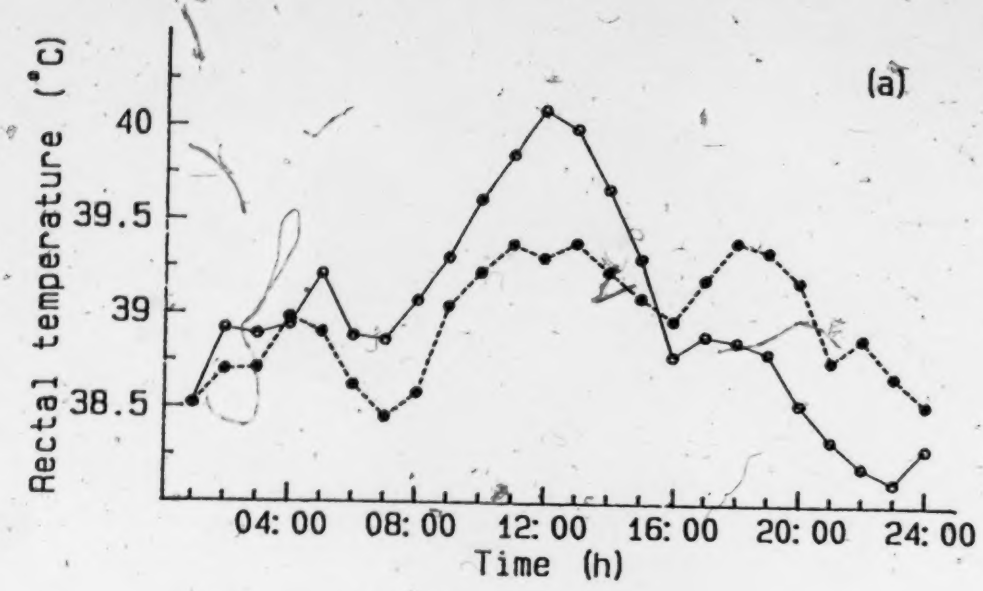




Fig. III.2. Hourly heart rates of lambs fed a control (o——o) or cimaterol-supplemented (o- - -o) ration on day 22 during (a) low-level (SEM 14) and (b) high-level (SEM 16) of dietary intake. Measurements for a pair-treated (Δ····Δ) group are also included at the higher intake.



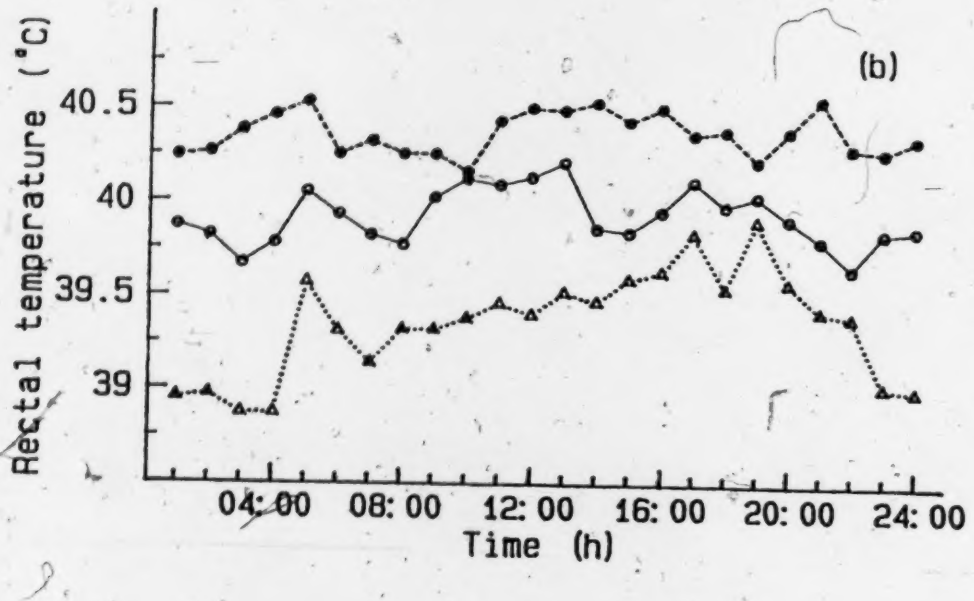




Fig. III.3. Hourly rectal temperatures of lambs fed a control (o——o) or cimaterol-supplemented (•– – –•) ration on day 22 during (a) low-level (SEM 0.4) and (b) high-level (SEM 0.6) of dietary intake. Measurements for a pair-treated (Δ····Δ) group are also included at the higher intake.

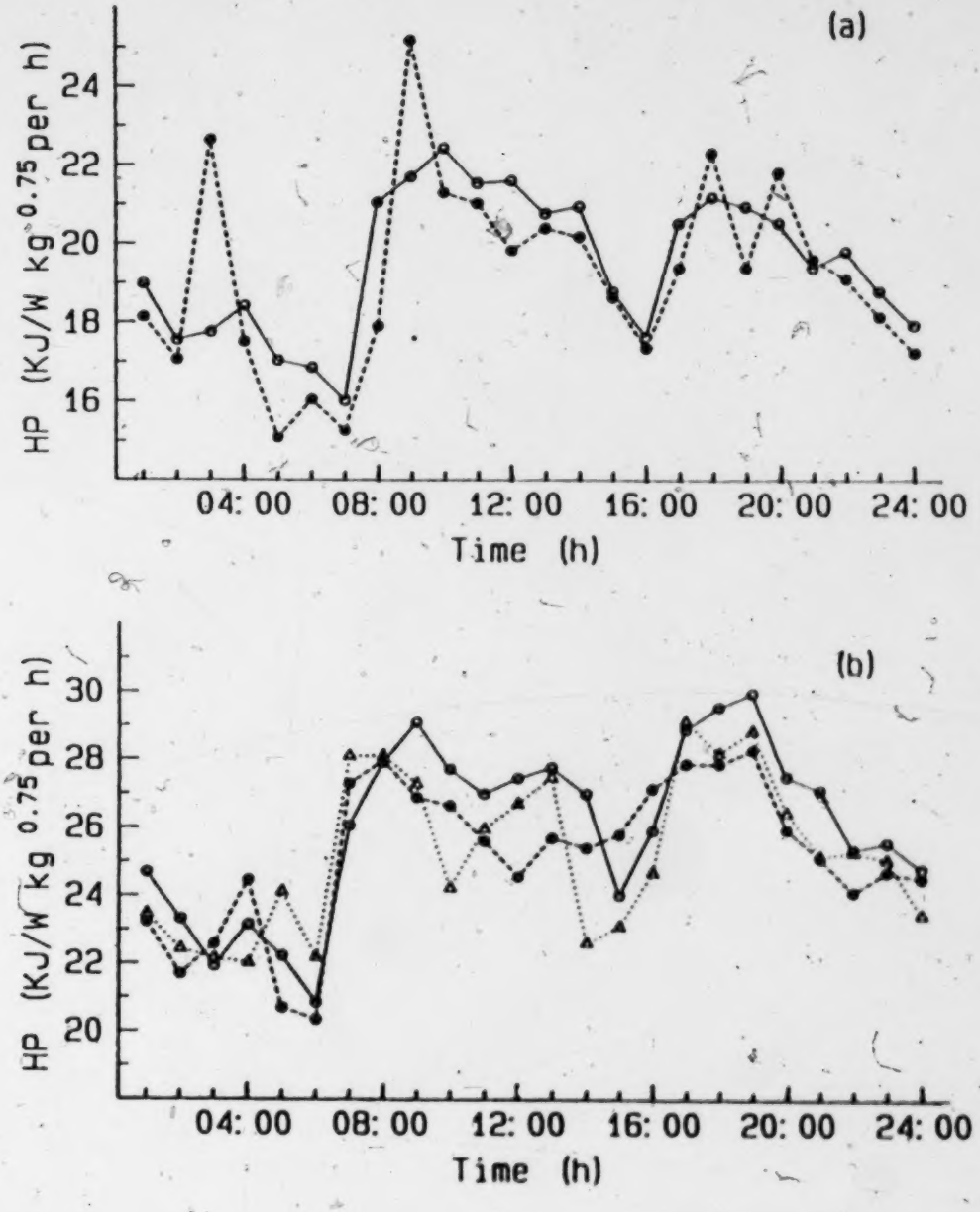



Fig. III.4. Hourly heat production (HP) of lambs fed a control (o——o) or cimaterol-supplemented (o- - -o) ration on day 22 during (a) low-level (SEM 2.0) and (b) high-level (SEM 2.5) of dietary intake. Measurements for a pair-treated (Δ·····Δ) group are also included at the higher intake.

# IV. GENERAL DISCUSSION AND CONCLUSIONS

## A. CARCASS CHARACTERISTICS AND CHEMICAL COMPOSITION

In the present study cimaterol-treated growing lambs had increased carcass to slaughter weight ratios and skeletal muscle weights compared to control animals. The cimaterol-treated lambs also had improved average daily gains over both the low and high dietary levels of intake.

Muscle weights. The three muscles selected for weight comparisons between the treatment groups in the present study, were chosen because of differences in their relative amounts of white, red and intermediate type fibers. Histological analysis indicates that the gastrocnemius and psoas major muscles have relatively less white and more red fibers than the semitendinosus muscle of sheep (Suzuki, 1976). The intermediate fiber content is similar in the three muscles. The proportion of fiber types in muscles will change with the nutrition and physical activity (Swatland, 1984) or stage of growth (Beermann et al. 1986) of animals. Maltin et al. (1987) showed that the $\beta$-agonist clenbuterol and tenotomy of the gastrocnemius muscle acted as synergists to stimulate hypertrophy of the fast glycolytic fibers in the rat soleus and plantaris muscles. Similarly, clenbuterol caused a hypertrophy of fast glycolytic fibers in the soleus muscle of rats with intact hindlimbs (Maltin et al. 1987). Moreover, in lambs treated for 56 days with cimaterol no changes were found in the proportion of fiber types in the longissimus dorsi and semitendinosus muscles although a greater

cross-sectional area of the white fibers indicated muscle mass increases were due to hypertrophy of predominately glycolytic tissues (Kim et al. 1987). In our study, there was a relative increase of about 0.44 in the weights of the gastrocnemius and psoas major muscles (with a larger proportion of red fibers) for the cimaterol-treated compared to control lambs, whereas the respective increase in the semitendinosus muscle was only 0.27 (Table II.4). This greater increase in the weights of muscles which characteristically have a lower anaerobic capacity is a surprising contradiction to what was expected based on the reported proportions of the three fiber types in these muscles (Suzuki, 1976). The differences in fat content would not account for the change in muscle weight since the protein:fat ratios were similar for the three muscles (Table II.5). The mechanism by which $\beta$-agonists mediate change in chemical composition and the proportion of fiber types making up the skeletal muscle mass of treated animals remains unknown and needs further study.

## B. PHYSIOLOGICAL RESPONSES

Physiological response and stress. The large within group variability for the physiological responses measured in this study negated significant differences between the three treatments (Table III.3). Nevertheless, it is the subjective opinion of the author, based on observations of the alertness and activity of the lambs during the experiment, that the cimaterol-treated animals showed a greater stress response to environmental stimuli. At the low level of dietary intake, erratic changes in heat production during

intravenous infusion at 03:00 and during feeding at 08:30 and 16:30 (Figure III.4 [a]) and a sustained post-feeding increase in heart rates (Figure III.2 [a]) indicates a greater physiological responsiveness for the cimaterol compared to control lambs. These responses suggest that chronic $\beta$-agonist treatment mimics the prolonged anxiety stress reflex (Axelrod & Reisine 1984).

Normal acute metabolic response to stress is brought about by increased sympathoadrenal tone. Typical of the characteristic catabolic-like response associated with acute stress is the net increase in blood flow to peripheral tissues and uptake and release of metabolites which occurs during short term clenbuterol supplementation (Rothwell et al. 1987 and Eisemann et al. 1988). However, animals which undergo extended periods of stress activate the pituitary-adrenal systems to acquire an adaptative resistance to these physiological effects (Axelrod & Reisine 1984) and blood flow to peripheral tissues has been shown to return to normal in chronically clenbuterol-treated rats (Rothwell et al. 1987). Adrenocorticotropin is released by activation of $\beta_2$-adrenoreceptors present in the anterior pituitary (Axelrod & Reisine 1984). Therefore, the anabolic activities of $\beta$-agonists may be partly due to glucocorticoids which appear to have a permissive role in the growth-promoting action of these agents.

Clenbuterol had no effect on growth or muscle weights in adrenalectomized rats but the repartitioning effect was reinstated when glucocorticoid activity was restored with dexamethasome treatment (Sharpe et al. 1986). In a study by Rothwell and Stock

(1988) of normal, clenbuterol-treated and untreated adrenalectomized rats, there were improvements in weight gains, suggesting a direct effect of β-agonists on lean body mass.

Acute metabolic changes such as increased thermogenesis may be transient whereas chronic effects like relatively high increases in heart rate in response to stressors could be sustained over longer periods of treatment with β-agonists. Buffering of a β-agonist stimulated homeorhetic metabolism is biologically sensible; the importance of this adaptability in livestock from a practical management standpoint is obvious. Frequent elevated heart rates might have dire consequences for the health of β-agonist treated animals, particularly when they are handled or moved to slaughter.

## C. MAINTENANCE REQUIREMENTS AND ENERGETIC EFFICIENCIES

Maintenance requirement. It is perhaps a bit perplexing that in this study we were unable to determine a difference in maintenance requirements between control and cimaterol-treated growing lambs. Yet, we did measure significant increases in the rate of daily gain and in carcass and muscle weights of the β-agonist treated groups. However, even slight shifts in metabolic rate, which were not measurable within the short analytical periods used in this study, can have tremendous consequence on growth over a longer period of time.

Estimates of maintenance for the treatment groups were within the normal range of values reported for growing lambs (ARC, 1980). Moreover, at the high dietary level the lambs may have been in a

realimentative state after the previous regime of lower energy intake. Therefore, the energy increments for maintenance of control and cimaterol-treated lambs were probably indicative of dietary energy intake requirements intermediate of the two dietary levels studied. Ferrell et al. (1986) demonstrated that growing lambs previously on a low plane of energy intake have a maintenance requirement during a subsequent high plane of energy intake which is less than animals which had continuously been on the higher plane of energy intake.

$Na^+,K^+$-ATPase-dependent respiration. The tendency for in vitro liver tissue $Na^+,K^+$-ATPase to be reduced by cimaterol treatment (Table II.3) could be a pertinent homeorhetic regulatory change in the metabolism of $\beta$-agonist treated lambs. $Na^+,K^+$ ion transmembrane exchange contributes markedly to maintenance energy expenditure (McBride and Milligan, 1985) and was recently shown to be highly correlated to the rate of protein synthesis in swine skeletal muscle (Adeola et al. 1989). However, the reduction in oxygen consumption for $Na^+,K^+$-ATPase-dependent respiration in the liver biopsies from our cimaterol-treated lambs (Table II.3) is a contradiction to what was expected. Presumably the increase in skeletal muscle accretion for the $\beta$-agonist treated lambs would have placed a greater metabolic load on their livers, resulting in an increase in energy expenditure from that organ. The liver makes a large contribution to energy expenditure, therefore, the similarity of the maintenance requirements determined for our cimaterol-treated and control lambs was surprising (Table III.4).

A reduction in energy expenditure for $Na^+,K^+$ ion transport

could be counterbalanced by an increase in substrate cycles, particularly those associated with glucose and triacylglycerol turnover. Rabkin and Blum (1985) reported that a substantial 0.26 portion of ATP production in incubated control rat hepatocytes is hydrolyzed for substrate cycling. The fructose 6-phosphate/ fructose 1,6-bisphosphate cycle being the largest consumer of the five cycles studied. The activity of enyzmes mediating flux through this cycle is determined by their state of phosphorylation. Therefore, they are likely to be modulated by $\beta$-agonist enhanced cAMP concentrations. The fructose 6-phosphate/fructose 1,6-bisphosphate cycling rate in the epitrochlearis muscles of rats has been shown to be increased about twelve-fold by the $\beta$-agonist isoprenaline (Challiss et al. 1984b). This cycle is also stimulated by a number of other $\beta$-adrenergic agonists, the effects being abrogated by the $\beta$-specific blocker propranolol (Challiss et al. 1984a). Likewise, the activity of the enyzmes catalyzing the glucose/glucose 6-phosphate, glycogen/glucose 6-phosphate, phosphoenolpyruvate/pyruvate/ oxaloacetate and acetate/acetyl-CoA cycles are dependent on phosphorylation (Rabkin and Blum, 1985). Elevation of the triacylglycerol/fatty acid cycle flux in isolated adipocytes of rats (Brooks et al. 1986) and in white and brown adipose tissue of mice in vivo (Brooks et al. 1983) has been shown to be elicited by $\beta$-adrenergic agents.

Changes in the flux through substrate cycles may be the primary metabolic events which result in an elevation of energy expenditure during periods of stress after exercise and feeding (Newsholme, 1986). These cycles allow an increased sensitivity and tractability

of metabolic control independent of large changes in metabolite or enzyme concentrations.

Energy costs of tissue deposition. The energy costs of protein and fat gain in non-ruminants are estimated to be about the same, approximately 53 KJ MEI/g (Pullar and Webster,1977). The 1.98 coefficient determined for $RE_p$ in the cimaterol lambs, when the cost of fat deposition is assumed to be 52 KJ MEI/g, suggests an energy cost of 47 KJ MEI/g for protein deposition. Although not significant ($P=0.19$) this coefficient does suggest a 0.50 efficiency for lean tissue deposition which is greater than the 0.10 to 0.40 reported by ruminant studies (Garrett, 1980). The energy cost of protein deposition in the cimaterol lambs (Table III.4) is also consistent with a reduction in rate of protein degradation rather than an increase in rate of protein synthesis as shown previously in rats (Reeds et al. 1986) and lambs (Bohorov et al. 1987). In the cimaterol group the cost of fat tissue deposition (51 KJ MEI/g determined from the 1.30 $RE_f$ coefficient, Chapter III) indicates normal ruminant lipogenesis from volatile fatty acids.

Beyond the shortcomings of regression analyses, the metabolizable energy partitioning data are consistent with predicted physiological changes in $\beta$-agonist treated animals. The cimaterol lambs were depositing more protein with a higher efficiency than their control counterparts.

In this study using regression analyses of energy data collected from control and cimaterol-treated lambs, it was concluded that the repartitioning effects of $\beta$-agonists are not necessarily brought about by large metabolic and physiological changes in treated

animals. Furthermore, there was not a significant change in the metabolic rate of cimaterol-treated lambs which confirms the homeostatic regulation with which metabolism is controlled.

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